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Please correct these transpositions of footnotes in Volume 27, No. 2 of the Iowa State College Journal of Science (January, 1953).

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1 a. Chairman of Committee, L. K. Arnold, Dept. of Chemical Engineering.
b. Doctoral Thesis No. 1157. Submitted March 14, 1951.

2 a. B.S., University of Shanghai, Shanghai, China, 1944.
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1 a. Chairman of Committee, Harley R. Willhelm, Dept. of Chemical Engineering.
b. Doctoral Thesis No. 1204. Submitted July 31, 1951.

2 a. B.S., Northwestern University, Evanston, Ill., 1947.
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1 a. Chairman of Committee, Joseph M. Keller, Dept. of Physics.
b. Doctoral Thesis No. 1211. Submitted August 25, 1951.

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SOLUTION BY DUAL INTEGRAL EQUATIONS OF A PLANE-STRAIN BOUSSINESQ PROBLEM FOR AN ORTHOTROPIC MEDIUM

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1. A Special Pair of Dual Equations. A solution for f(t) of the dual integral equations

$$\int_0^\infty t^s f(t) J_p(ut)\, dt = g(u), \quad 0 < u < 1, \tag{1}$$

$$\int_0^\infty f(t) J_p(ut)\, dt = 0, \quad u > 1, \tag{2}$$

has been given in explicit form by Busbridge [1] subject to the restrictions that g(u) be integrable in (0, 1) and that

$$s > -2, \quad -p - 1 < s - 1/2 < p + 1. \tag{3}$$

In this paper the case will arise where $s = -1$, $p = -1/2$ and $\sqrt{u}\, g(u)$ is a polynomial in u. Thus the condition (3) is violated and it will now be shown that the Busbridge formula will fail unless the coefficients of the abovementioned polynomial satisfy a certain relation.

If $\sqrt{u}\, g(u) = u^n$ the Busbridge formula, after some simplification, yields as a "solution" $f(t) = f_n(t)$, where

$$f_n(t) = \left[\sqrt{2}\, \Gamma\left((n+1)/2\right) \Big/ \Gamma(n/2)\right] t^{\frac{s}{2}} \int_0^1 x^n J_1(tx)\, dx. \tag{4}$$

By direct substitution it is found that the second of the dual equations is satisfied but not the first; in fact, one finds that

$$\int_0^\infty t^{-1} f_n(t) J_{-\frac{1}{2}}(ut)\, dt = u^{-\frac{1}{2}}\left(u^n - \gamma_n/\sqrt{\pi}\right), \tag{5}$$

where

$$\gamma_n = \Gamma\left((n+1)/2\right) \Big/ \Gamma\left((n+2)/2\right). \tag{6}$$

It is now evident from this that the Busbridge formula has failed to provide a solution. In particular, it should be mentioned that the right hand member of (5) vanishes when $n = 0$.

Now take

$$\sqrt{u}\, g(u) = c_0 + c_1 u + c_2 u^2 + \ldots + c_m u^m, \tag{7}$$

where the c's are constants.

If $f_n(t)$ is still defined by (4), it will now be shown that

$$f(t) = c_1 f_1(t) + c_2 f_2(t) + \ldots + c_m f_m(t) \tag{8}$$

is a solution of the dual equations under consideration, provided there is a certain linear relation among the c's, namely,

$$-\sqrt{\pi}\, c_0 = c_1\gamma_1 + c_2\gamma_2 + \ldots + c_m\gamma_m. \tag{9}$$

For, the second of the dual equations clearly remains satisfied, and the first is also satisfied now, since it follows from (5) that

$$\int_0^\infty t^{-1} \sum_{n=1}^{m} f_n(t)\, J_{-\frac{1}{2}}(ut)\, dt = u^{-\frac{1}{2}}\left[\sum_1^m c_n u^n - \sum_1^m c_n\gamma_n \Big/ \sqrt{\pi}\right] = g(u).$$

2. Statement of the Problem. Consider a semi-infinite elastic medium with a plane boundary. Using rectangular cartesian coordinates (x,y,z) with the origin in the boundary, choose the x-axis so that it is directed normally into the medium. In this paper it is assumed that the medium is orthotropic, as defined by I. S. Sokolnikoff [2] with the coordinate planes as planes of elastic symmetry and that, in addition, the physical properties of the medium are unaltered in description when the y- and z-axis are interchanged.

The main object of this paper is to determine the stress in the medium when the plane boundary is indented by a rigid punch which is in the form of a long cylinder with generators parallel to the z-axis. All sections by planes perpendicular to the z-axis will then be identical in behavior so that the problem becomes two-dimensional, that is, one of plane-strain. It is, therefore, sufficient to confine attention to the plane z = o and to consider the stress at the point (x, y).

Further, it will be assumed that the punch is well lubricated, that it is in contact with the medium over the strip $-a < y < a$, and that the curve of contact in the (x, y) plane is symmetrical about the x-axis. More precisely, the curve of contact will be assumed to be of the polynomial form

$$x = g(y) = \sum_0^m A_n |y|^n, \qquad |y| < a. \tag{10}$$

The method of solution via dual integral equations fails in the apparently simple case where g(y) is a constant. This exceptional case is therefore separately considered and the results so obtained are used to complete the solution of the more general problem.

Throughout this paper it will be assumed that the body forces in the medium are negligible and that there is no external load apart from that due to the punch. Also, the strains are supposed to be small so that the classical theory of elasticity may be used.

3. The Potential Function ϕ. Plane-strain phenomena in an orthotropic medium of the type described above have been studied by R. H. Tripp and D. L. Holl [3] and this section is a summary of the first part of their paper with a slight modification in the notation.

Four elastic constants E_x, E_y, σ_x, σ_y, which may be called generalized Young's moduli and Poisson's ratios, are used to specify the elastic properties of such an orthotropic substance. They are connected by one relation $E_x/E_y = \sigma_y/\sigma_x$, and it is convenient to introduce two more elastic constants k and K defined by

$$k^2 = E_y/E_x = \sigma_x/\sigma_y, \tag{11}$$

$$K^2 (1 - \sigma^2_y) = k^2 (1 - \sigma^2_y k^2). \tag{12}$$

For the sake of simplicity E_y, σ_y, will henceforth be written as E, σ, respectively without the subscript.

Under plane-strain conditions, the equilibrium equations and the compatibility relations are satisfied if the stress components are derivable from a potential function ϕ in accordance with the equations

$$\tau_{xx} = \partial^2\phi/\partial y^2, \quad \tau_{yy} = \partial^2\phi/\partial x^2, \quad \tau_{xy} = -\partial^2\phi/\partial x\partial y, \tag{13}$$

where $\phi(x,y)$ satisfies the differential equation

$$\frac{\partial^4\phi}{\partial x^4} + (1 + K^2) \frac{\partial^4\phi}{\partial x^2\partial y^2} + K^2 \frac{\partial^4\phi}{\partial y^4} = 0. \tag{14}$$

The displacement components (u, v) are then given by the equations

$$\begin{aligned} E(\partial u/\partial x) &= k^2(1-\sigma^2k^2)\,\tau_{xx} - k^2\sigma(1+\sigma)\,\tau_{yy}, \\ E(\partial v/\partial y) &= (1-\sigma^2)\,\tau_{yy} - k^2\sigma(1+\sigma)\,\tau_{xx}, \\ G\,[\partial u/\partial y + \partial v/\partial x] &= \tau_{xy}, \end{aligned} \tag{15}$$

where G is a shear modulus defined by the relation

$$E/G = k^2 (1-\sigma^2 k^2 + \sigma^2 + \sigma) + 1-\sigma^2 + k^2\sigma + k^2\sigma^2. \tag{16}$$

For the isotropic case $E_x = E_y = E$, $\sigma_x = \sigma_y = \sigma$ and $K = k = 1$.

4. The Equivalent Dual Integral Equations. The problem on hand, as stated in Section 2, is equivalent to the task of solving the differential equation (14) subject to the boundary conditions

$$\tau_{ij} \longrightarrow 0 \text{ as } x^2 + y^2 \longrightarrow \infty, \tag{17}$$

$$\left[\tau_{xy}\right]_{x=o} = 0 \text{ identically in } y, \tag{18}$$

$$\left[u\right]_{x=o} = g(y), \quad |y| < a, \tag{19}$$

$$\left[\tau_{xx}\right]_{x=o} = 0, \quad |y| > a. \tag{20}$$

The procedure henceforth is mainly formal, but once a solution is obtained it can be verified. The main defect is that the uniqueness of the solution is not assured mathematically.

Suppose $\phi(x, y)$ is the desired solution and let $\phi(x, \beta)$ be its Fourier transform relative to y, namely,

$$\bar{\phi}(x, \beta) = \int_{-\infty}^{\infty} \phi(x, y)\, e^{i\beta y} dy.$$

By making the assumption, suggested by condition (17) that $\partial^n\phi/\partial y^n \longrightarrow 0$ as $|y| \longrightarrow \infty$, for n = 1,2,3, it follows that the differential equation (14) for $\phi(x, y)$ implies that the Fourier transform $\bar{\phi}(x, \beta)$ satisfies the ordinary differential equation

$$\left[D^4 - (1 + K^2)\beta^2 D^2 + K^2\beta^4\right] \bar{\phi} = 0, \tag{21}$$

where $D = d/dx$ and β is regarded as a parameter.

In view of the condition (17) one chooses a solution of (21) of the form

$$\bar{\phi}(x, \beta) = A\, e^{-|\beta|x} + B\, e^{-K|\beta|x}, \tag{22}$$

where A and B depend on β.

Now from the relation

$$\tau_{xy} = -\partial^2\phi/\partial x \partial y$$

it follows that

$$\bar{\tau}_{xy} = i\beta(d\bar{\phi}/dx),$$

where bars over a symbol indicate Fourier transforms. The inversion of the last equation yields

$$\tau_{xy} = (1/2\pi i) \int_{-\infty}^{\infty} \beta|\beta| \left(Ae^{-|\beta|x} + KBe^{-K|\beta|x}\right) e^{-i\beta y}\, d\beta, \qquad (23)$$

so that condition (18) will be satisfied if $A + KB = 0$.

Therefore, if one chooses $\bar{\phi}$ to be of the form

$$\bar{\phi}(x, \beta) = B(\beta) \left(K e^{-|\beta|x} - e^{-K|\beta|x}\right), \qquad (24)$$

then it remains only to determine $B(\beta)$ so that the last two boundary conditions (19), (20) are also met. From the relation

$$\tau_{xx} = \partial^2\phi/\partial y^2$$

it follows, by taking the Fourier transform of each side, with respect to y, that

$$\bar{\tau}_{xx} = -\beta^2 \bar{\phi}(x, \beta),$$

provided

$$(\partial\phi/\partial y - i\beta\phi)\, e^{i\beta y}$$

tends to zero as y tends to $\pm\infty$. This latter condition will be assumed to hold. The inversion theorem applied to the last equation above now yields

$$\tau_{xx} = -(1/2\pi) \int_{-\infty}^{\infty} \beta^2 B(\beta) \left[Ke^{-|\beta|x} - e^{-K|\beta|x}\right] e^{-i\beta y}\, d\beta. \qquad (25)$$

Therefore, the condition (20) will be met if

$$\int_{-\infty}^{\infty} \beta^2 B(\beta)\, e^{-i\beta y}\, d\beta = 0, \quad |y| > a. \qquad (26)$$

In order to meet the remaining boundary condition it is desirable to express u in terms of ϕ. This is done by first seeking v. Making similar assumptions as before on the behavior of ϕ as $|y|$ tends to infinity, one obtains from the second equation (15), after some simplification

$$E\, \bar{v}(x, \beta) = i\beta B(\beta)\, (1 + \sigma) \left[b\, e^{-|\beta|x} - b' e^{-K|\beta|x}\right], \qquad (27)$$

where

$$b = K(1 - \sigma + k^2\sigma), \quad b' = K^2(1 - \sigma) + k^2\sigma. \qquad (28)$$

The Fourier inversion theorem now yields

$$E\,v = (1/2\pi)(1+\sigma)\int_{-\infty}^{\infty} \beta B(\beta)\left[b\,e^{-|\beta|x} - b'e^{-K|\beta|x}\right]e^{-i\beta y}\,d\beta. \quad (29)$$

In a similar way the third of equations (15) together with (27) yields

$$2\pi G u = K\int_{-\infty}^{\infty} |\beta| B(\beta)\left[c\,e^{-|\beta|x} + c'e^{-K|\beta|x}\right]e^{-i\beta y}\,d\beta, \quad (30)$$

where

$$\begin{aligned} c &= 1 + (G/E)(1+\sigma)(1-\sigma+k^2\sigma), \\ c' &= -1 + (G/E)(1+\sigma)\left[K^2(1-\sigma) + k^2\sigma\right]. \end{aligned} \quad (31)$$

Hence, the remaining boundary condition (19) will be satisfied if

$$K(c+c')\int_{-\infty}^{\infty} |\beta| B(\beta)\, e^{-i\beta y}\,d\beta = 2\pi G\, g(y), \quad |y| < a. \quad (32)$$

It is now clear that the desired form for $\bar{\phi}(x, \beta)$ is given by equation (24) where $B(\beta)$ satisfies the pair of dual integral equations (32), (26). As there is symmetry about the x-axis in the problem on hand it follows from equation (24) and the definition of the Fourier transform that $B(\beta)$ is an even function so that, after setting $t = a\beta$, $Y = y/a$, the dual equations may be written

$$\begin{aligned} &\int_0^{\infty} t^{-1} f(t)\, J_{-\frac{1}{2}}(Yt)\,dt = Mg(aY)/\sqrt{Y}, \quad |Y| < 1, \\ &\int_0^{\infty} f(t)\, J_{\frac{1}{2}}(Yt)\,dt = 0, \quad Y > 1, \end{aligned} \quad (33)$$

where

$$M = \frac{\sqrt{2\pi}\,G\,a^2}{K(c+c')}, \quad (34)$$

$$f(t) = t^{5/2} B(t/a). \quad (35)$$

So the mixed boundary value problem of this paper is reduced to the task of solving the dual equations (33) for $f(t)$. The components of stress and displacement can now be obtained directly from $f(t)$. To do this, first consider equation (25). Putting $X = x/a$ and remembering that $B(\beta)$ is an even function related to $f(t)$ by equation (35), one obtains from (25)

$$\tau_{xx} = \frac{-1}{\pi a^3}\int_0^{\infty} t^{-\frac{1}{2}} f(t)\left(Ke^{-Xt} - e^{-KXt}\right)\cos Yt\,dt. \quad (36)$$

In similar fashion one finds

$$\tau_{yy} = \frac{K}{\pi a^3} \int_0^\infty t^{-\frac{1}{2}} f(t)(e^{-Xt} - K e^{-KXt}) \cos Yt \, dt, \tag{37}$$

$$\tau_{xy} = \frac{-K}{\pi a^3} \int_0^\infty t^{-\frac{1}{2}} f(t)(e^{-Xt} - e^{-KXt}) \sin Yt \, dt, \tag{38}$$

$$u = \frac{K}{\pi Ga^2} \int_0^\infty t^{-\frac{3}{2}} f(t)(c e^{-Xt} + c' e^{-KXt}) \cos Yt \, dt, \tag{39}$$

$$v = \frac{(1+\sigma)}{\pi Ea^2} \int_0^\infty t^{-\frac{3}{2}} f(t)(b e^{-Xt} - b' e^{-KXt}) \sin Yt \, dt. \tag{40}$$

5. Indentation by a Rectangular Block. The object of this section is to solve the problem stated in Section 2 when $g(y)$ is a constant. For this case it appears that the dual integral equations do not have a solution. Hence, the procedure in this section is rather indirect.

Suppose the boundary $x = 0$ is subjected to the loading

$$q(y) = \begin{cases} W/(\pi \sqrt{a^2 - y^2}), & |y| < a, \\ 0, & |y| > a, \end{cases} \tag{41}$$

where W is clearly the total applied load. It will appear later that this loading produces a constant deflection under the load and so yields a solution to the problem of this section.

Now let $\phi(x, y)$ be the solution of the differential equation (14) which satisfies the boundary conditions

$$\tau_{ij} \to 0 \text{ as } x^2 + y^2 \to \infty, \tag{42}$$

$$[\tau_{xy}]_{x=0} = 0 \text{ identically in } y, \tag{43}$$

$$[\tau_{xx}]_{x=0} = -q(y). \tag{44}$$

The results of Section 3 shows that, to meet conditions (42) and (43), the Fourier transform of ϕ with respect to y should be of the form

$$\bar{\phi}(x, \beta) = B(\beta) \left[K e^{-|\beta| x} - e^{-K|\beta| x} \right]. \tag{45}$$

To satisfy the remaining boundary condition (44) it is necessary to choose $B(\beta)$ so that

$$\int_0^\infty \beta^2 B(\beta) \cos y\beta \, d\beta = \begin{cases} W/\left[(K-1) \sqrt{a^2 - y^2} \right], & |y| < a, \\ 0, & |y| > a. \end{cases}$$

Upon inversion this gives

$$\beta^{n} B(\beta) = \frac{W}{K-1} J_0(\beta a). \tag{46}$$

Taking the Fourier transforms of equations (13), substituting for $\bar{\phi}(x, \beta)$ from (45), (46) and then using the inversion theorem, one obtains

$$\tau_{xx} = \frac{-WK}{\pi(K-1)} \int_0^\infty J_0(\beta a) \left[e^{-\beta x} - (1/K) e^{-K\beta x} \right] \cos y\beta \, d\beta,$$

$$\tau_{yy} = \frac{WK}{\pi(K-1)} \int_0^\infty J_0(\beta a) \left[e^{-\beta x} - K e^{-K\beta x} \right] \cos y\beta \, d\beta,$$

$$\tau_{xy} = \frac{WK}{\pi(K-1)} \int_0^\infty J_0(\beta a) \left[-e^{-\beta x} + e^{-K\beta x} \right] \sin y\beta \, d\beta.$$

It is convenient now to introduce the complex variables z and s defined by

$$z = x + iy, \qquad s = Kx + iy. \tag{47}$$

Hence, by making use of the integral

$$\int_0^\infty e^{-at} J_0(\gamma t) \, dt = \left(a^2 + \gamma^2\right)^{-\frac{1}{2}}, \quad \mathcal{R}\, a > |\mathcal{I}\gamma|,$$

one obtains for the stress components at an interior point

$$\tau_{xx} = \frac{WK}{\pi(K-1)} \mathcal{R}\left[\frac{-1}{\sqrt{a^2+z^2}} + \frac{1/K}{\sqrt{a^2+s^2}} \right], \tag{48}$$

$$\tau_{yy} = \frac{WK}{\pi(K-1)} \mathcal{R}\left[\frac{1}{\sqrt{a^2+z^2}} - \frac{K}{\sqrt{a^2+s^2}} \right], \tag{49}$$

$$\tau_{xy} = \frac{WK}{\pi(K-1)} \mathcal{R}\left[\frac{1}{\sqrt{a^2+z^2}} - \frac{1}{\sqrt{a^2+s^2}} \right]. \tag{50}$$

The values of the stress components at points of the boundary can most easily be found separately or from (48)-(50) by a continuity argument. In any case, the results agree with the boundary conditions (43), (44).

The determination of the displacement is more awkward. The method used in Section 4, leading to equations (39), (40) will fail here since the Fourier inversion theorem will be found to be inapplicable. So one substitutes the values of the stress just obtained into the primary relations (15). Hence,

$$\frac{\pi(K-1)E}{KW} \frac{\partial u}{\partial x} = \mathcal{R}(-\alpha z_1 + K\alpha' s_1), \tag{51}$$

$$\frac{\pi(K-1)E}{KW} \frac{\partial v}{\partial y} = \mathcal{R}(\gamma z_1 - \gamma' s_1), \tag{52}$$

$$\frac{\pi(K-1)G}{KW} \left(\frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \right) = \mathcal{I}(z_1 - s_1), \tag{53}$$

where the newly introduced symbols are defined by

$$z_1 = (a^2 + z^2)^{-\frac{1}{2}}, \quad s_1 = (a^2 + s^2)^{-\frac{1}{2}},$$

$$\alpha = k^2(1 - \sigma^2 k^2) + k^2\sigma(1 + \sigma),$$

$$\alpha' = k^2(1 - \sigma^2 k^2)/K^2 + k^2\sigma(1 + \sigma),$$

$$\gamma = (1 - \sigma + k^2\sigma)(1 + \sigma),$$

$$\gamma' = (1 + \sigma)\left[K(1 - \sigma) + (k^2\sigma)/K\right].$$

Integration of equations (51) and (52) yields

$$\frac{\pi(K - 1)Eu}{KW} = \mathcal{R}\left[-\alpha \sinh^{-1}(z/a) + \alpha' \sinh^{-1}(s/a)\right] + F(y), \qquad (54)$$

$$\frac{\pi(K - 1)Ev}{KW} = \mathcal{I}\left[\gamma \sinh^{-1}(z/a) - \gamma' \sinh^{-1}(s/a)\right] + G(x),$$

where the inverse hyperbolic functions are to be interpreted as their principal values. By considerations of symmetry it is clear that v must vanish when $y = 0$ and this implies that $G(x)$ is identically zero. When the above expressions for u and v are substituted into equation (53) there results

$$(G/E)\left[F'(y) + \mathcal{I}\left\{(\alpha + \gamma)\, z_1 - (\alpha' + K\gamma')s_1\right\}\right] = \mathcal{I}(z_1 - s_1). \qquad (55)$$

It is readily seen that

$$\alpha + \gamma = \alpha' + K\gamma' = E/G$$

so that the equation (55) is valid if, and only if, $F'(y) = 0$. Therefore, $F(y)$ is a constant, say D, and so the displacement components are given by equations (54) with $F(y)$ replaced by D and $G(x)$ by zero. If one sets $x=0$ in the expression for v in (54), it is found that the quantity in the square brackets is purely imaginary for $|y| < a$, showing that the deflection is constant under the load. Therefore, the problem solved in this section is precisely the one propounded at the outset.

The results for the isotropic case can be derived from those just obtained by letting K tend to unity and using l' Hospital's theorem.

It should be observed that (54) gives infinite values for u, v at infinity. This is a known defect of the classical theory for plane-strain deformations and is noted by S. Timoshenko [4], p.88 and D. L. Holl [5], p.48. The constant D may be chosen so that an arbitrarily selected point on the surface is taken as a standard reference point relative to which the other displacements are measured and the formulas for the displacements are considered to be valid only over a certain finite range. If D is chosen to be

$(2\pi/K)(K-1)(1-\sigma^2)$ log 2 then the results for the isotropic case agree with those obtained by M. Sadowsky [6].

6. Symmetric Indentation of Polynomial Type. This section is devoted to the main problem as stated in Section 2, the equation of the indenting punch being

$$x = g(y) = \sum_0^m A_n |y|^n , \quad |y| < a, \tag{56}$$

It should be observed that A_1, A_2, ... are geometric contatns of the punch whereas A_0 is not. It is now convenient to introduce two new constants c_0 and c_1 by the definitions

$$c_0 = -(1/\sqrt{\pi}) \sum_1^m A_n a^n \gamma_n , \quad c_1 = A_0 - c_0, \tag{57}$$

where γ_n is given by equation (6).

The stress and displacement in the medium are derived from g(y) by means of linear operations so that the problem being considered may be solved by superposing the solutions of two indentation problems, the first being that due to a punch whose equation is

$$x = h(y) = c_0 + \sum_1^m A_n |y|^n , \quad |y| < a, \tag{58}$$

and the second that due to the penetration of a rectangular block to a depth c_1. The second problem has already been solved in Section 5 so that it remains to deal with the case where the curve of contact is given by equation (58) with c_0 given by equation (57).

From Section 4 it follows that the stress and displacement are obtainable from f(t) by the relations (36)-(40), where f(t) is the solution of the dual integral equations

$$\int_0^{\infty} t^{-1} f(t) \; J_{-\frac{1}{2}}(Yt)\, dt = M\, h(aY)/\sqrt{Y}, \quad |Y| < 1, \tag{59}$$

$$\int_0^{\infty} f(t) \; J_{-\frac{1}{2}}(Yt)\, dt = 0, \quad Y > 1,$$

where

$$M = \frac{\sqrt{2\pi}\; G\, a^s}{K(c + c')} . \tag{60}$$

The dual integral equations (59) are exactly of the type for which an explicit solution was developed in Section 1. Therefore,

$$f(t) = M \sum_1^m A_n a^n f_n(t), \tag{61}$$

where

$$f_n(t) = \frac{-2\sqrt{2}\,\gamma_n}{n}\, t^{\frac{n}{2}} \int_0^1 u^n J_1(tu)\, du. \tag{62}$$

The stress and displacement can now be obtained from equations (36)-(40). In order to express the answers in simple form it is convenient to introduce some new quantities by the definitions

$$Z = X + iY, \qquad S = KX + iY, \tag{63}$$

$$P_{mn} = \int_0^1 u^n du \int_0^\infty t^m J_1(ut)\, e^{-Zt}\, dt, \tag{64}$$

$$Q_{mn} = \int_0^1 u^n du \int_0^\infty t^m J_1(ut)\, e^{-St}\, dt, \tag{65}$$

$$\gamma'_n = \frac{\Gamma\left(\frac{n+1}{2}\right)}{\Gamma\left(\frac{n}{2}\right)} = \frac{n}{2}\gamma_n \tag{66}$$

$$L = \frac{2G}{a\sqrt{\pi}\,K(c+c')} = \frac{2E}{a\sqrt{\pi}\,(1-\sigma^2)(K^2-1)K}. \tag{67}$$

The integrals (64),(65) are reguired only for m = 0 or 1. They can be evaluated by using the standard integrals

$$\int_0^\infty e^{-pt} J_1(ut)\, dt = u^{-1}\left[1 - p\Big/\sqrt{u^2 + p^2}\right],$$

$$\int_0^\infty t\, e^{-pt} J_1(ut)\, dt = u\,(u^2 + p^2)^{-\frac{3}{2}},$$

these being valid for $p > 0$. Hence, one obtains

$$P_{on}(X, Y) = \int_0^1 u^{n-1}\left[1 - Z(u^2 + Z^2)^{-\frac{1}{2}}\right] du,$$

$$P_{1n}(X, Y) = \int_0^1 u^{n+1}(u^2 + Z^2)^{-\frac{3}{2}}\, du,$$

$$Q_{on}(X, Y) = \int_0^1 u^{n-1}\left[1 - S(u^2 + S^2)^{-\frac{1}{2}}\right] du,$$

$$Q_{1n}(X, Y) = \int_0^1 u^{n+1}(u^2 + S^2)^{-\frac{3}{2}}\, du.$$

After substituting for f(t) from (61)-(63) into equations (36)-(40) one now obtains

$$\tau^*_{xx} = \mathcal{R}\sum_1^m LA_n\gamma'_n a^n (K P_{1n} - Q_{1n}),$$

$$\tau^*_{yy} = -\mathcal{R}\sum_1^m LKA_n\gamma'_n a^n (P_{1n} - KQ_{1n}),$$

$$\tau^*_{xy} = -\mathcal{R}\sum_1^m LKA_n\gamma'_n a^n (P_{1n} - Q_{1n}),$$

$$G u^* = (-aKL)\mathcal{R}\sum{}'_{n}^{m} A_n \gamma'_n a^n \,(cP_{on} + c'Q_{on}),$$

$$E v^* = a(1+\sigma)\mathcal{I}\sum{}'_{n}^{m} A_n \gamma'_n a^n (b\,P_{on} - b'Q_{on}),$$

the first three equations being restricted by the condition that X be positive in order to avoid divergent integrals. Asterisks have been used above on the symbols τ and u as these are not the final components of stress and displacement. If, in the problem of the penetration of a rectangular block to a depth of c_1, the various quantities be distinguished by the superscript (o), then the stress and displacement components for the main problem of this chapter are given by

$$\tau_{ij} = \tau^*_{ij} + \tau^{(o)}_{ij}, \qquad u_i = u_i^* + u_i^{(o)}.$$

Also, if W is the total load applied to the boundary,

$$W = W^* + W^{(o)},$$

The expression for τ^*_{xx} after an integration by parts, may be combined with the expression for $\tau^{(o)}_{xx}$ obtained from (48) to yield

$$\tau_{xx} = \mathcal{R}\left[\left(\frac{W^{(o)}}{a\pi(K-1)} + L\sum{}'_{n}^{m} A_n \gamma'_n a^n\right)\left(\frac{-K}{\sqrt{1+Z^2}} + \frac{1}{\sqrt{1+S^2}}\right)\right] \tag{68}$$

$$+ L\mathcal{R}\sum{}'_{n}^{m} A_n \gamma'_n a^n \int_0^{\prime} n u^{n-1}\left(\frac{K}{\sqrt{u^2+Z^2}} - \frac{1}{\sqrt{u^2+S^2}}\right)du.$$

Hence, τ_{xx} is finite at the point X = 0, Y = 1, if and only if

$$W^{(o)} = -a\pi(K-1)L\sum{}'_{n}^{m} A_n \gamma'_n a^n.$$

Therefore, the final expressions for the stress components become

$$\begin{aligned}
\tau_{xx} &= \mathcal{R}\sum{}'_{n}^{m} LA_n \gamma'_n a^n n \int_0^{\prime} u^{n-1}\left(\frac{K}{\sqrt{u^2+Z^2}} - \frac{1}{\sqrt{u^2+S^2}}\right)du,\\
\tau_{yy} &= -\mathcal{R}\sum{}'_{n}^{m} LKA_n \gamma'_n a^n n \int_0^{\prime} u^{n-1}\left(\frac{1}{\sqrt{u^2+Z^2}} - \frac{K}{\sqrt{u^2+S^2}}\right)du,\\
\tau_{xy} &= -\mathcal{I}\sum{}'_{n}^{m} LKA_n \gamma'_n a^n n \int_0^{\prime} u^{n-1}\left(\frac{1}{\sqrt{u^2+Z^2}} - \frac{1}{\sqrt{u^2+S^2}}\right)du.
\end{aligned} \tag{69}$$

These integrals are elementary so that the stress components are expressible in closed form in terms of simple functions. Also, an inspection of these formulas show immediately that the boundary conditions on τ_{xx} and τ_{yy} are satisfied.

It is important to determine the quantity a in terms of the total load W and the other constants of the problem. To do this, one first writes down

$$\tau_{xx}(0, Y) = \sum_{1}^{m} LA_n\gamma'_n a^n n \int_{Y}^{1} \frac{(K-1)\, u^{n-1}}{\sqrt{u^2 - Y^2}}\, du, \quad 0 < Y < 1.$$

Hence, one finds that

$$W = -2a \int_0^1 \tau_{xx}(0, Y)\, dY = W^{(o)}.$$

This then yields

$$W(1-\sigma^2)K(K+1) + 2\sqrt{\pi}\, E \sum_{1}^{m} A_n\gamma'_n a^n = 0$$

from which a can be determined.

The components of displacement are found by simple superposition of the results of this section with equation (54). To check the boundary condition on u, one finds that, if X = 0 and $0 < Y < 1$,

$$u(0, Y) = c_1 - \frac{aKL}{G}(c+c') \sum_{1}^{m} A_n\gamma'_n a^n \left[\frac{1}{n} - Y \int_0^Y \frac{u^{n-1}\, du}{\sqrt{Y^2 - u^2}}\right],$$

which, with the help of (31), (57), (66), simplifies to the relation

$$u(0, Y) = c_1 + c_0 + \sum_{1}^{m} A_n a^n Y^n, \quad 0 \leq Y \leq 1.$$

The right-hand member of this equation is precisely g(y) so that the boundary condition on u is verified.

It has now been verified that the final expressions for the stress and displacement do, in fact, satisfy all the boundary conditions. To complete the verification of the solution one can substitute the expressions (69) for the stress components into the equilibrium equations

$$(\partial/\partial x)\, \tau_{xx} + (\partial/\partial y)\, \tau_{xy} = 0,$$

$$(\partial/\partial x)\, \tau_{xy} + (\partial/\partial y)\, \tau_{yy} = 0,$$

and the compatibility relation (14). An inspection of (69) shows immediately that the equilibrium equations are satisfied. Also, the compatibility relation (14) can be written in the form

$$\left[(\partial^2/\partial x^2) + K(\partial^2/\partial y^2)\right] (\tau_{xx} + \tau_{yy}) = 0,$$

and this is clearly satisfied since,

$$\tau_{xx} + \tau_{yy} = L(K^2 - 1)\mathcal{R} \sum_{1}^{m} A_n\gamma'_n a^n n \int_0^1 \frac{u^{n-1}\, du}{\sqrt{u^2 + S^2}}.$$

The results for the isotropic case can be deduced from the foregoing by letting K tend to unity. The formulas so obtained are in essential agreement with those given by Sneddon [7] although his treatment contains puzzling features such as the condition imposed concerning "zero resultant force on the boundary."

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PARASITES OF THE EUROPEAN CORN BORER IN IOWA[1]

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INTRODUCTION

The first work in Iowa with parasites of the European corn borer, *Pyrausta nubilalis* (Hbn.), began in 1926, sixteen years before the corn borer reached the state (29), when the parasite *Microbracon brevicornis* (Wesm.) was reared on the smartweed borer, *Pyrausta ainsliei* Heinrich, and released in smartweed patches with the hope that it would become established and then transfer its attack to the European corn borer if and when the latter arrived (19, 3).

The State Entomologist of Iowa, with cooperation of the Agricultural Experiment Station and the U. S. Bureau of Entomology and Plant Quarantine, in the fall of 1943 started to introduce the insect enemies of the pest into the state (14, 20). Annually since then the State Department of Agriculture has made funds available for procuring parasite adults for colonization. The parasites have been reared at the Bureau's laboratory at Moorestown, New Jersey, and shipped to Iowa. Most releases have been made by state personnel, including entomologists of the State Department of Agriculture, Experiment Station and Extension Service — a few releases were made in eastern Iowa from 1944 through 1948 by Bureau personnel located at the Muscatine, Iowa, Laboratory, and the small number of releases in 1950 were made by the Bureau personnel at the European Corn Borer Laboratory at Ankeny.

The annual report of the State Entomologist in the *Iowa Yearbook of Agriculture* has recorded annually the number of each species and the township in which they were released (20, 21, 25, 27, 28). Similarly the Bureau has summarized the numbers of each species released in Iowa and other states (5, 6, 7, 9, 17).

Studies by the cooperating agencies have been continued annually to learn whether the introduced parasites became established initially and persisted after establishment and also to obtain information regard-

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[1] Journal Paper No. J-2118 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1193. In large part based on "Insect Parasites of *Pyrausta* Species in Iowa" by Carl C. Blickenstaff, a thesis on file in the Iowa State College Library, submitted to the Graduate Faculty, Iowa State College, in partial fulfillment of the requirements for the degree of Master of Science.

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ing spread and abundance. From these studies records were obtained also on parasite species indigenous to Iowa which were found attacking the European corn borer. The state agencies made all of the collections, except a few in the vicinity of Muscatine, which were made by the Bureau from 1944 through 1949, and in 1950 state and federal agencies worked together.

Some of the collections of hibernating host larvae taken each fall from 1944 through 1947 were processed at the Experiment Station as a part of the state corn borer project. All other collections from 1944 through 1949 were processed at the Moorestown, N. J., or the Toledo, Ohio, Laboratory of the Bureau, utilizing the methods described by Baker *et al.* (10). Collections in 1950 were processed at the European Corn Borer Laboratory at Ankeny.

SOURCES OF PARASITES RELEASED IN IOWA

Nine species of exotic parasites have been released in Iowa, including the larvaevorid, *Lydella stabulans grisescens* R. D.; three ichneumonids, *Campoplex alkae* Ell. & Sacht., *Horogenes punctorius* (Roman), and *Phaeogenes nigridens* Wesm.; four braconids, *Apanteles thompsoni* Lyle, *Chelonus annulipes* Wesm., *Macrocentrus gifuensis* Ashm., and *Microgaster tibialis* Nees., and one eulophid, *Sympiesis viridula* (Thoms), previously called *Eulophus viridulus.* The numbers of each species released each year are shown in Table 1.

Four of the nine parasites, *L. stabulans grisescens, H. punctorius, M. gifuensis* and *C. annulipes,* were reared from hibernating corn borer larvae collected in eastern states where they were plentiful. The methods described by Baker, *et al.* (10) were used except that after 1946 the borers for *L. stabulans grisescens* production were isolated in shell vials in the same manner as those for other species. Most of the *L. stabulans grisescens* came from the vicinity of Moorestown, New Jersey; *H. punctorius* from East Hartford, Connecticut; *M. gifuensis* from East Hartford and Taunton, Massachusetts, and *C. annulipes* from Taunton. The number of *C. annulipes* obtained from field collections was small in 1949 and 1950, and the method of laboratory rearing described by Bradley (11) was used to produce them in greater numbers.

P. nigridens for colonization were reared from collections of first brood European corn borer pupae taken from fields in Concord, Lincoln and Waltham, Massachusetts, in July and August, 1945.

Laboratory reared, hibernating *S. viridula* pupae were obtained from the Dominion Parasite Laboratory, Belleville, Ontario, Canada through the courtesy of A. B. Baird. Adults were reared from the pupae at Toledo in 1945 and 1946 and at Moorestown in 1947 and 1948.

Cocoons of hibernating *C. alkae* and *M. tibialis* were collected during the fall and winter in Europe by the Paris, France, laboratory of the Bureau's Division of Foreign Parasite Introduction. The cocoons were shipped to Moorestown, where they were stored and later incubated to produce adults according to the methods described by Arbuthnot and Baker (4). *A. thompsoni* adults were reared at Moorestown from hiber-

TABLE 1

NUMBERS OF EUROPEAN CORN BORER PARASITES RELEASED IN IOWA TO DECEMBER 31, 1951

Year	*Apanteles thompsoni*	*Campoplex alkae*	*Chelonus annulipes*	*Horogenes punctorius*	*Lydella stabulans grisescens*	*Macrocentrus gifuensis*	*Microgaster tibialis*	*Phaeogenes nigridens*	*Sympiesis viridula*	Total
1944	0	0	490	3,133	8,090	122,115	0	0	0	133,828
1945	0	0	1,494	5,110	5,368	46,602	0	500	5,536	64,610
1946	0	0	526	3,067	12,578	143,051	0	0	4,984	164,206
1947	0	0	1,472	2,906	25,489	257,020	0	0	3,969	290,856
1948	0	0	135	856	1,449	38,188	0	0	3,997	44,625
1949	0	0	2,563	917	960	82,821	0	0	0	87,261
1950	0	279	7,471	485	548	21,175	33	0	0	29,991
1951	691	719	2,309	642	269	21,167	1,044	0	0	26,841
TOTAL	691	998	16,460	17,116	54,751	732,139	1,077	500	18,486	842,218

nating European corn borer larvae taken in Europe incidental to the collecting of *C. alkae* and *M. tibialis* cocoons.

COLONIZATION TECHNIQUES

Release sites and species to be liberated in Iowa were selected by general procedures described by Baker, *et al.* (10). The earliest releases (those made in 1944) were made in eastern parts of the state, which the borer first invaded and where high populations first developed. As the pest spread and its numbers increased westward in later years, parasite releases were made farther west and in 1947 some were released near the western boundary of the state. Four principal parasite study localities were established in Muscatine, Marshall, Harrison, and Hancock counties, located respectively in the eastern, central, western and northern parts of the state, and in 1948, 1949, and 1950, parasite releases have been concentrated in these localities.

M. gifuensis was the most plentiful parasite available for release and in the first three years, about three colonies per county were released in eastern Iowa. *L. stabulans grisescens* was available in about the proportion of one colony (500 adults) to three of *M. gifuensis* (2,000 adults each) and usually no more than one colony was released per county. The other species were less plentiful and were released at greater intervals between colonies.

Group colonizations of *C. annulipes* were made in 1949 and 1950, similar to those made in 1939 in Connecticut and Massachusetts and in 1940 in New York (10).

Recoveries of *M. gifuensis* in Iowa and other middle western states showed a preponderance of males in several instances, especially from collections taken later than the year of release. Inasmuch as unfertilized females produce only male offspring this indicated mating was not satisfactorily accomplished in the field, and it was thought that dispersion of adults from the release site might provide insufficient concentrations of progeny to facilitate contact between the sexes. One possible means of providing higher progeny populations was the releasing of groups of colonies close enough to one another that dispersion from them would overlap. This type of release was made during the period 1948-51. Another possible means was continuation of releases even after establishment of the species was accomplished to provide released adults in addition to the progeny present in the field. This was done in Britt Township, Hancock County, in 1949 and 1950, where *M. gifuensis* showed survival in 1948 from a release made there in 1947. Insufficient time has elapsed since these techniques were used in making releases to evaluate them in comparison with the releasing of colonies at one site or discontinuing releases after initial establishment was shown.

TECHNIQUES USED TO DETERMINE THE FIELD STATUS OF PARASITES

Collections of European corn borers to determine the status of parasites fall into four main categories: (1) Those taken in the immediate vicinity of release sites to determine if parasites became estab-

lished in the same year and if they survived in any later year. (2) Those taken systematically around release sites with location of sampling points determined by use of either a polar or rectangular coordinate arrangement to determine survival, rate and direction of dispersion, and changes in abundance. (3) Those taken systematically from extensive areas, without regard to the location of release sites to determine the extent of parasitization in large areas. (4) Collections taken where parasites had not been released, which sometimes provided information on the dispersion of released species. All methods frequently gave data on the attack by native parasites on the corn borer.

The experiment station studies involved records of parasitization taken throughout the year. All other information from 1944 through 1949 was on hibernating host larvae except at Muscatine in the spring of 1947, 1948, and 1949 when host pupae from overwintered borers were observed. In 1950, overwintered larvae, and pupae developed from them, were collected in the spring; and first and second brood larvae and pupae from numerous localities and areas throughout the state were collected and reared for parasite data. Many of the larvae collected during the summer in 1950 hibernated. They were overwintered in cold storage and incubated in the spring.

All of the accumulated information on parasites of the European corn borer in Iowa is presented in the following discussions.

FIELD STATUS OF IMPORTED PARASITES IN IOWA

Lydella stabulans grisescens R. D.

Colonization

The biology of this species has been discussed in considerable detail elsewhere (10, 36). It has been colonized in 87 localities, in 52 counties in the State as shown in Table 2, and 54,751 adults have been released.

Field Status

Lydella stabulans grisescens is the most widely established and abundant parasite of the European corn borer in Iowa and has been taken in 143 townships in 42 counties in the state. It is noteworthy that when a collection was taken in a later year at a site where *grisescens* had been recovered, the species again appeared in the collection except in four localities, Fredericka Township, Bremer County; Eldora Township, Benton County; Green Township, Fremont County; and College Township, Linn County. Of these the collections in Fremont County were taken about four miles from the release site where it was recovered in 1948, the year in which it was released.

The location of release sites and places where *Lydella* was recovered are shown in Figure 1. In 12 counties, releases but no recoveries were made. Releases were made in 1947 in eight of these 12 counties and collections taken as follows failed to produce this parasite: Allamakee 1947, 1948, and 1949; Audubon 1947; Chickasaw 1947; Decatur

TABLE 2

Lydella stabulans grisescens ADULTS RELEASED IN IOWA TO DECEMBER 31, 1951

County	Township	No. released
Year—1944		
Cedar	Dayton	491
"	Sugar Creek	412
Clinton	Comanche	484
"	Orange	488
Des Moines	Flint River	490
Dubuque	White Water	455*
Henry	Canaan	496
Jackson	South Fork	424*
"	Washington	491
Johnson	West Lucas	496*
Jones	Cass	439*
Lee	Jefferson	491*
Linn	College	491*
Louisa	Wapello	487*
Muscatine	Sweetland	498
Scott	Winfield	466
Washington	Oregon	491*
	TOTAL	8,090
Year—1945		
Benton	Eldorado	487
Dubuque	White Water	484*
Grundy	Washington	490
Henry	Marion	480*
Iowa	Lenox	486*
Jackson	South Fork	492*
Johnson	West Lucas	478*
Lee	Jefferson	488*
Louisa	Wapello	495*
Poweshiek	Warren	489*
Scott	Butler	499*
	TOTAL	5,368
Year—1946		
Benton	Fremont	492
Black Hawk	Big Creek	485
Bremer	Jackson	477
Buchanan	Fremont	461*
Cedar	Fremont	467*
Clayton	Cox Creek	484*
Delaware	South Fork	321*
Dubuque	White Water	394*
Fayette	Jefferson	482*
Grundy	Melrose	490*
Henry	Scott	470*
Iowa	Lenox	496*
Jackson	South Fork	409*
Jasper	Clear Creek	183*
Johnson	Liberty	499*
Jones	Cass	431*
Keokuk	Sigourney	485*
Lee	Jefferson	463*
Linn	College	493*
"	Jackson	469
Year—1946 (Cont.)		
Louisa	Columbus City	497
Mahaska	Monroe	490
Marshall	Liscomb	491
Poweshiek	Warren	490*
Scott	Butler	487*
Story	Lafayette	198
Tama	Otter Creek	485
Washington	Oregon	489*
	TOTAL	12,578
Year—1947		
Allamakee	Franklin	494
Audubon	Hamlin	490
Buchanan	Fremont	327*
"	Hazelton	458
"	Sumner	450
Chickasaw	Jacksonville	465
Clayton	Cox Creek	492*
Davis	Cleveland	454
"	Lick Creek	901
"	Prairie	482
Decatur	Franklin	498
Delaware	Delaware	497
"	Honey Creek	485
"	South Fork	497*
Dubuque	Prairie Creek	495
"	Table Mound	470
Emmet	Twelve Mile Lake	475
Fayette	Jefferson	450*
"	Union	475
"	West Field	440
Guthrie	Valley	495
Harrison	Harrison	498*
Henry	Jackson	495
"	Marion	491*
"	Scott	490*
Howard	Vernon Springs	490
Iowa	Hartford	487
"	Lenox	988*
"	Tray	492
Jefferson	Black Hawk	478
"	Center	470
"	Locust Grove	475
Keokuk	English River	488
"	Sigourney	992*
"	Washington	488
Marion	Indiana	492
Palo Alto	Emmetsburg	497
Shelby	Center	495
Union	Jones	491
Van Buren	Des Moines	496
"	Farmington	499
"	Van Buren	499

TABLE 2 (Continued)

County	Township	No. released
Year—1947 (Cont.)		
Wapello	Highland	492
"	Pleasant	492
"	Washington	465
Washington	English River	500
"	Franklin	494
"	Oregon	493*
Webster	Lost Grove	497
Winneshiek	Decorah	475
	TOTAL	25,489
Year—1948		
Fremont	Green	405
Harrison	Harrison	560*
Plymouth	Elgin	484
	TOTAL	1,449
Year—1949		
Hancock	Britt	480
Harrison	Harrison	480*
	TOTAL	960
Year—1950		
Harrison	Harrison	548*
Year—1951		
Warren	Washington	269
	GRAND TOTAL	54,751

* Released here in more than one year.

1949; Emmet 1949; Marion 1949; Palo Alto 1947; and Union 1949. In Fayette County no recoveries were made from a collection taken in Jefferson Township in 1946, the year in which it was released there. The following year releases were made in three townships and the only collection taken thereafter was in the summer of 1950, when it was recovered. Both releases and collections were made annually in Harrison Township, Harrison County from 1947 through 1950 but none was recovered until the last year. In Britt Township, Hancock County, none was recovered from a 1949 collection following a release that year, but it was recovered in 1950. No collections have been taken in Davis and Jefferson Counties, in each of which three releases were made in 1947.

In some other counties, collections were not taken from every one of the release localities, but *L. stabulans grisescens* was recovered in the county. Table 3 shows a complete list of recoveries in the state including all of those obtained through observations on overwintered host larvae in the winter of 1949-1950, and the first brood in the summer of 1950.

Collections of hibernating borers were taken in four or five years at six release sites and provide some data on *L. stabulans grisescens* after it became established. The percentage parasitized was less than ten when the fly was first taken in five localities but in Liscomb Township, Marshall County, it was higher. No release was made after initial establishment was shown in Cox Creek Township, Clayton County; Comanche Township, Clinton County; Flint River Township, Des Moines County; and Liscomb Township, Marshall County, but

two later releases were made in White Water Township, Dubuque County, and Oregon Township, Washington County. No advantage to the fly is indicated from releases made after it became established since the percentages of borers parasitized did not increase faster there than where no post recovery release was made.

An ecological study of the European corn borer was started in Muscatine County in 1944. Two study areas, *K* and *L*, were established and each one consisted of 12 one-mile-square sections arranged as shown in Figure 2, numbered consecutively from 1 to 12, beginning with the

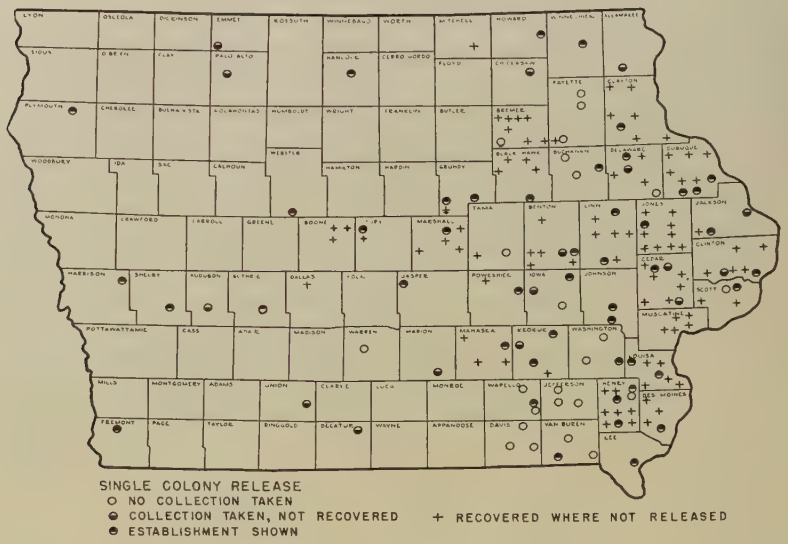

FIG. 1. — Localities in Iowa where *Lydella stabulans grisescens* has been released and the recovery records obtained.

upper left and progressing toward the right in each row of four sections. *L. stabulans grisescens* was released in the northeast quarter of Section 11, Area *K*, on June 22, 1944. One sample of approximately fifty borers was taken from each section in the fall of 1944, and in each later year two samples were taken, each consisting of about twenty-five borers. The total borers observed was 610, 575, 602, 523, 550, 521, and 472 in *K* and 568, 484, 587, 563, 567, 598, and 476 in *L*, respectively, in 1944, 1945, 1946, 1947, 1948, 1949, and 1950. The percentages of borers parasitized in the sections are shown in Table 4. *L. stabulans grisescens* was recovered from Sections 7, 8, and 10, all adjoining Section 11, in Area *K* in 1944. In 1945 it was taken in all but two sections, 1 and 9, in Area *K*, when the percentage exceeded 14 in the four sections 7, 8, 11, and 12 and was greater than 10 per cent in only one other

section (No. 2). Every one of the 12 sections in area *K* produced this parasite in 1946, and it was taken in four eastern sections of area *L*, more than seven miles from the release site. In 1947, 1948, 1949, and 1950 recoveries were made in every one of the twenty-four sections in the two areas. The 1947 collections, taken three years after the release was made, showed dispersion to at least nine miles from the release site.

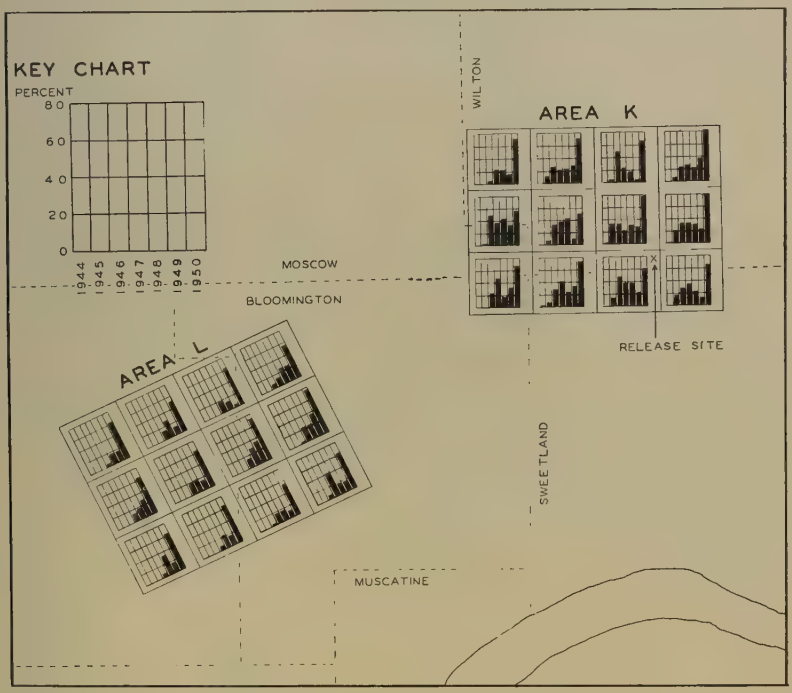

FIG. 2.—Dispersion and abundance of *Lydella stabulans grisescens* in two study areas in Muscatine County, Iowa.

The percentage of borers parasitized in Area *K* increased annually from 1.2 in 1944 to a maximum of 32.3 in 1947, decreased in 1948 and 1949 to 24.8 in 1949, then increased to 68.6 in 1950. In Area *L*, parasitization increased from 1.4 per cent in 1946 to 22.8 per cent in 1948, decreased to 19.6 per cent in 1949, and in 1950 increased to 63.4.

A survey was made in the fall of 1948 and 1949 to determine parasitization over an extensive area in eastern Iowa by taking a 50-borer sample from about every other one of the townships, without regard to parasite release sites. Data from ten surveyed counties are grouped into three areas, and two counties are shown separately, in Table 5.

TABLE 3

Lydella stabulans grisescens Recoveries in Iowa

County	Township	Number of borers observed: 1944	1945	1946	1947	1948	1949	1950	Percentage of borers parasitized: 1944	1945	1946	1947	1948	1949	1950
Benton	Eldorado			34						*	5.9				
	Florence						18							16.3	
	Fremont			19		51					0*		27.5		
	Iowa			12		57	94	21			0		3.5	18.1	14.3
	Jackson						3							33.3	
	Kane					68	87	28					1.5	31.0	21.4
	Union					71	17						29.6	23.5	
Black Hawk	Barclay						3							66.6	
	Bennington							7[1]							28.6
	Big Creek			41	53						4.9*	1.9			
	Cedar Falls			73			84	186[1]			0			0	4.8
	Mt. Vernon							13[1]							15.4
	"							3							33.3
Boone	Des Moines					57		319[1]					0		0.3
	Dodge							25[1]							4.0
	Harrison						544	218[1]						0	3.2
Bremer	Douglas							12[1]							8.3
	"							9							11.1
	Franklin						5							20.0	
	Fredericka						10							10.0	
	Fremont							4							50.0
	LeRoy						97	268[1]						6.1	2.6
	"							16							25.0
	Maxfield							23[1]							13.0
	Polk							254[1]							2.0
	Warren							37[1]							18.9
Buchanan	Fremont			59							15.3*	*			

TABLE 3 (Continued)

County	Township	Number of borers observed:							Percentage of borers parasitized:						
		1944	1945	1946	1947	1948	1949	1950	1944	1945	1946	1947	1948	1949	1950
Cedar	Dayton	96		48					7.3*		0				
	Inland					53	46						58.5	26.1	
	Pioneer		19	58			36			0	0			19.4	
	Red Oak					57	48						38.6	31.3	
	Rochester					55	29						16.4	20.7	
	Springdale					61	25						52.5	24.0	
	Springfield					56	47						62.5	19.1	
	Sugar Creek	440	4	42					0.2*	0	0				
Clayton	Cox Creek			48	48	54	46				0*	10.4*	14.8	21.7	
	Elk					54	48						0	6.3	
	Girard					47	43						0	14.0	
	Grand Meadow					54	53						0	5.7	
	Highland					38	49						0	2.0	
	Millville					50	45						0	22.2	
	Read			48		49	47				0		0	6.4	
Clinton	Brookfield					64	22						53.1	31.8	
	Comanche	434	37	47	87		40		0.9*	10.8	29.8	41.4		25.0	
	Deep Creek					49	41						26.5	14.6	
	DeWitt					58	45						34.5	53.3	
	Eden		33	95		91				0	0		33.0		
	Olive					53	50						34.0	28.0	
	Waterford					63	49						52.4	20.4	
Dallas	Beaver							7							14.3
Delaware	Delhi					48	43						0	23.3	
	Elk					42	49						2.4	8.2	
	Honey Creek			46		47	49				0	*	25.5	24.5	
	Prairie					46	42						0	9.5	
Des Moines	Danville						40							15.0	
	Flint River	88	14	45		57			3.4*	7.1	2.2		28.1		
	Franklin					54	46						9.3	17.4	
	Washington		23	42		60	45			0	0		33.3	8.9	

TABLE 3 (Continued)

County	Township	Number of borers observed:							Percentage of borers parasitized:						
		1944	1945	1946	1947	1948	1949	1950	1944	1945	1946	1947	1948	1949	1950
Dubuque	Concord					57	50						3.5	6.0	
	Dodge					50	46						16.0	8.7	
	Liberty					42	47						0	17.0	
	Peru					54	48						5.6	12.5	
	Prairie Creek			52	93						0	3.3*			
	Table Mound			48	58*	58	52				0	0*	15.5	1.9	
	Taylor					62							6.5		
	Vernon						38							42.1	
	White Water			51	110	56	46		*	*	3.9*	24.5	25.0	28.3	
Fayette	Oran							20[1]							10.0
	"							9							44.4
Fremont	Green					152							1.3		
Grundy	Felix							165							18.2
	Melrose			135				57			0*				28.1
	Washington			416						*	1.2				
Guthrie	Valley				41							2.4*			
Hancock	Britt					65	247	211					0	0*	0.9
Harrison	Harrison				46	175	221	288[1]				0*	0*	0*	2.5*
Henry	Baltimore						69	210[1]						7.2	35.2
	"							1							100.0
	Center					57	47						31.6	12.8	
	Jackson			42		46	51	1			0	*	8.7	15.7	100.0
	Jefferson					45	73	8[1]					4.4	19.2	12.5
	"							1							100.0
	Marion			45				22[1]		*	15.6		*		22.7
	"							9							33.3
	New London							5[1]							20.0
	"							1							100.0

TABLE 3 (Continued)

County	Township	Number of borers observed: 1944	1945	1946	1947	1948	1949	1950	Percentage of borers parasitized: 1944	1945	1946	1947	1948	1949	1950
Henry (Cont.)	Salem					52	100	49[1]					1.9	14.0	44.9
	Scott			50			38	197[1]			4.0*	*		26.3	32.0
	"							3							0
	Tippecanoe					51	18	4[1]					9.8	5.6	20.0
	"							1							0
	Wayne					56	52	159[1]					28.6	26.9	24.5
	"							3							33.3
Howard	Vernon Springs						46							2.2	
Iowa	Lenox			45			28			*	0*	*		67.9	
Jackson	Monmouth			44	42	44					0	2.4	31.8		
	South Fork			524					*	*	1.7*				
Jasper	Clear Creek			7			48				14.3*			4.2	
Johnson	Liberty			47			25				0*			24.0	
	West Lucas			45					*	*	13.3*				
Jones	Cass		20	48		42			*	0	2.1*		19.0		
	Castle Grove					48	48						18.8	16.7	
	Fairview						47							14.9	
	Greenfield					55	46						49.1	26.1	
	Hale						45							35.6	
	Madison					46							50.0		
	Oxford					48	47						22.9	10.6	
	Richland					49	48						26.5	4.2	
	Rome					53							32.1		
	Scotch Grove						36							30.6	
Keokuk	English River						3					*		66.7	
	Jackson						2							50.0	
	Sigourney			20			3				0*	*		33.3	

TABLE 3 (Continued)

County	Township	Number of borers observed:							Percentage of borers parasitized:						
		1944	1945	1946	1947	1948	1949	1950	1944	1945	1946	1947	1948	1949	1950
Lee	Jefferson	93		42					0*	*	2.4*				
Linn	Bertram						44							29.5	
	Brown						17							58.8	
	College	93		45					1.1*		0*				
	Jackson			131			45				0*			15.6	
	Marion			13		250					0		11.2		
	Otter Creek						49							20.4	
	Spring Grove						40							12.5	
Louisa	Columbus City			49							6.1*				
	Concord					50	34						4.0	26.5	
	Eliot						34							5.9	
	Grandview					51	43						9.8	4.7	
	Jefferson					57							21.1		
	Morning Sun					75	51						50.7	5.9	
	Wapello			140					*	*	3.6				
Mahaska	Garfield					56	46						0	4.3	
	Monroe			12		48	8				8.3*		12.5	37.5	
	Richland						44							4.5	
	White Oak					19	42						0	9.5	
Marshall	LeGrande					52	46						1.9	32.6	
	Liscomb			78	197	159	88	75			16.7*	16.8	20.1	20.4	13.3
	Taylor						49							26.5	
	Vienna						77							24.7	
	Washington					45	46						0	2.2	
Mitchell	Burr Oak				35		189					0		2.1	

TABLE 3 (Continued)

County	Township	Number of borers observed: 1944	1945	1946	1947	1948	1949	1950	Percentage of borers parasitized: 1944	1945	1946	1947	1948	1949	1950
Muscatine	Bloomington	229	222	245	227	247	245	208	0	0	7.3	23.3	20.6	20.0	63.9
	Lake	385	309	388	377	366	398	293	0	0	0.2	14.3	23.5	20.4	67.2
	Moscow	50	50	51	45	47	49	37	0	2.0	49.0	35.6	42.6	32.6	51.4
	Sweetland	443	230	258	215	228	210	162	9.2*	18.7	33.3	34.9	28.5	23.3	74.1
	Wilton	261	248	247	222	229	220	208	0	5.2	23.9	26.6	25.8	23.2	72.6
Plymouth	Elgin	.....	.....	.....	.....	105	232	.....	.....	.....	.....	.....	4.8*	0.4	.....
Poweshiek	Sheridan	.....	.....	27	.....	216	.....	.....	.....	.....	0	.....	0.5	.....	.....
	Warren	.....	.....	.....	43	.....	.....	.....	.....	*	*	4.7	.....	.....	.....
Scott	Butler	.....	.....	51	.....	.....	.....	.....	.....	.....	23.5*	.....	.....	.....	.....
	Lincoln	.....	.....	45	.....	.....	.....	.....	.....	.....	6.7	.....	.....	.....	.....
Shelby	Center	.....	.....	.....	.....	.....	40	.....	.....	.....	.....	*	.....	10.0	.....
Story	Franklin	.....	.....	.....	.....	.....	80	6	.....	.....	.....	.....	.....	0	16.7
	Lafayette	.....	.....	9	.....	.....	40	1	.....	.....	0*	.....	.....	7.5	0
Tama	Indian Village	.....	43	13	.....	.....	40	.....	.....	0	15.4	.....	.....	7.5	.....
Van Buren	Des Moines	.....	.....	.....	.....	.....	1	.....	.....	.....	.....	*	.....	100.0	.....
Wapello	Pleasant	.....	.....	14	.....	.....	51	.....	.....	.....	0*	.....	.....	3.9	.....
Washington	Oregon	441	.....	143	.....	.....	50	.....	2.7*	.....	7.7*	*	.....	16.0	.....
Webster	Lost Grove	.....	.....	.....	.....	30	50	.....	.....	.....	.....	*	3.3	0	.....
Winneshiek	Decorah	.....	.....	.....	.....	.....	45	.....	.....	.....	.....	*	.....	6.7	.....

* Released here this year.
[1] This is a first brood collection, all others were hibernating borers.

Area 1 includes Clayton, Delaware, and Dubuque Counties, in which the first releases of *Lydella* were made in 1946, 1945, and 1944, respectively. Of eight samples taken each year in Clayton, the northernmost county surveyed, one sample in 1948 and seven in 1949, produced this parasite and the percentage of borers parasitized increased from 2.0 to 9.6. All four of the 1949 Delaware County collections produced this parasite, whereas it was produced from only two of four in 1948. Also, the percentage of borers attacked was more than twice as great in 1949. In Dubuque County only one sample (1948) failed to produce *L. grisescens* in the two years, 1948-1949, and parasitization increased from 10.6 to 15.6 between these years. Altogether nineteen samples were taken in Area 1 in each of the two years, and the percentage parasitized was more than twice as great in 1949 (13.2 per cent) as in 1948 (6.3 per cent).

Area 2 included Cedar, Clinton, and Jones Counties in 1948 and these three and Linn County in 1949, in all of which *L. stabulans grisescens* was first released in 1944. Every one of the samples taken in the two years produced this parasite, but the percentage of borers parasitized was much lower in 1949 than in 1948 in the three counties surveyed in both years. Parasitization was high in 1948 when it was 38.5 per cent in the three-county area, and even the 15.1 per cent lower parasitization (23.4) in 1949 was high compared with the European records for this species (36), and higher than the seven-year average percentage (20.6) in New Jersey where field collections were made to provide adult flies for colonization in Iowa.

Area 3 includes Des Moines, Henry, and Louisa Counties where *Lydella* was first released in 1944. Only one sample, from Henry County in 1949, failed to show this species among the 25 collections taken in the area in the two years. The percentage parasitized in 1949 was lower than in 1948 in each county with the greatest difference in Louisa, which was the highest in 1948 and lowest in 1949.

Among six samples taken each year in Mahaska County where this parasite was released in 1946, only one in 1948 and four in 1949 produced the fly and the percentage increased from 2.7 to 4.8 between these years. *Grisescens* was released in Marshall County in 1946, and in this county only three samples were taken each year. One and two of them produced the parasite in 1948 and 1949, respectively. The parasitization of all borers observed increased sharply from 0.7 per cent in 1948 to 12.2 in 1949.

Borers taken in seasonal history and population studies on the host in 1950 from five areas in the state were reared in the laboratory for parasite data. *L. grisescens* was prevalent in collections from Henry County in the Southeast and the Black Hawk — Bremer County area in the Northeast. The earliest, semi-weekly collection in Henry County was taken July 22 and the parasite was reared from it. The first corn borer pupae and the first parasite puparia were found in the field on July 27. Corn borer pupation was last found on August 19 and the last emergence on August 25, when the last collection was taken. There were 61

borers in the August 19 and August 25 collections and 26.2 per cent produced parasites when incubated after collection and 13 borers hibernated. The 13 borers were incubated after overwintering in cold storage and 4 or 6.6 per cent of the original number, produced *L. stabulans grisescens*. A first brood host population survey was made in the period

TABLE 4

Lydella stabulans grisescens IN TWO STUDY AREAS IN MUSCATINE COUNTY, IOWA

Area and section	Percentage of borers parasitized in:						
	1944	1945	1946	1947	1948	1949	1950
Area *K*							
North row							
1	0	0	4.2	23.2	22.2	14.9	72.5
2	0	10.4	26.0	21.7	22.7	28.9	72.1
3	0	3.9	50.0	23.9	17.1	4.5	66.7
4	0	6.4	24.0	25.6	22.0	37.5	81.4
Center row							
5	0	2.0	49.0	35.6	42.6	32.6	56.8
6	0	5.9	33.3	38.6	42.8	9.1	51.2
7	2.0	33.3	34.0	21.1	30.4	27.1	76.7
8	1.4	20.4	32.7	36.2	30.2	22.0	78.4
South row							
9	0	0	23.9	46.3	17.4	31.0	66.7
10	2.0	7.5	29.4	41.9	23.1	32.6	74.4
11	0	14.6	46.0	39.1	37.8	20.6	60.0
12	0	17.6	27.4	34.1	21.4	12.2	65.2
TOTAL	1.2	9.9	30.1	32.3	27.6	24.8	68.6
Area *L*							
Northwest row							
1	0	0	0	4.4	16.3	16.0	63.4
2	0	0	0	14.0	27.9	11.1	52.9
3	0	0	0	17.6	13.1	4.0	63.4
4	0	0	6.5	12.8	26.0	25.5	56.1
Center row							
5	0	0	0	14.0	20.0	28.6	64.1
6	0	0	0	19.1	21.4	15.7	64.1
7	0	0	0	14.9	29.4	35.4	73.0
8	0	0	1.9	23.4	24.4	38.2	77.8
Southeast row							
9	0	0	0	6.4	34.7	15.6	62.9
10	0	0	0	7.1	23.4	15.7	65.8
11	0	0	1.8	16.7	17.0	13.2	55.6
12	0	0	5.8	41.5	19.6	17.3	63.8
TOTAL	0	0	1.4	15.6	22.8	19.6	63.4

August 7-14, and the 50 borers collected were 30.0 per cent parasitized. A fall population survey provided 19 hibernating larvae and 36.8 per cent of them were parasitized.

Semi-weekly first brood borer collections were started on July 25 in the Black Hawk-Bremer area. The first parasite puparia was found in the field on July 28, six days before host pupation started on August 4. A first brood population survey was made in the period August

TABLE 5

THE ABUNDANCE OF *Lydella stabulans grisescens* ON HIBERNATING CORN BORERS IN IOWA IN AREAS SURVEYED IN THE FALL OF 1948 AND 1949

Area and County	Number of townships: Sampled 1948	Number of townships: Sampled 1949	Number of townships: Yielding parasites 1948	Number of townships: Yielding parasites 1949	Number of borers studied 1948	Number of borers studied 1949	Percentage of borers parasitized 1948	Percentage of borers parasitized 1949
Area 1								
Clayton	8	8	1	7	402	379	2.0	9.6
Delaware	4	4	2	4	183	183	7.1	16.4
Dubuque	7	7	6	7	379	327	10.6	15.6
TOTAL	19	19	9	18	964	889	6.3	13.2
Area 2								
Cedar	5	6	5	6	282	231	45.7	23.8
Clinton	6	6	6	6	378	247	39.2	28.7
Linn	.....	5	.....	5	.....	195	.....	23.1
Jones	7	7	7	7	341	317	31.7	19.0
TOTAL	18	24	18	24	1,001	990	38.5	23.4
Area 3								
Des Moines	3	3	3	3	171	131	24.0	13.7
Henry	6	5	6	4	307	195	15.0	14.9
Louisa	4	4	4	4	233	162	24.5	9.9
TOTAL	13	12	13	11	711	488	20.3	12.2
Area 4								
Mahaska	6	6	1	4	226	228	2.7	4.8
Area 5								
Marshall	3	3	1	2	148	131	0.7	12.2

5-25 and 6.6 per cent of the 241 borers were parasitized by *L. stabulans grisescens*. A fall population survey provided 73 borers of which 21.9 per cent were parasitized.

Resume of the Status of *Lydella stabulans grisescens* in Iowa

L. stabulans grisescens is widely established in the eastern half of Iowa as shown by recoveries in 20 counties as follows: Benton, Black Hawk, Bremer, Cedar, Clayton, Clinton, Delaware, Des Moines, Henry, Dubuque, Jackson, Jones, Keokuk, Linn, Louisa, Mahaska, Marshall, Muscatine, Poweshiek, and Scott. Within the area circumscribed by these counties its status has not been determined in Buchanan, Fayette, Iowa, Johnson, Tama, and Washington nor in several counties bordering the area. Some extension of dispersion beyond the area of more or less continuous distribution in the eastern part of the state is shown by recoveries in Boone, Dallas, and Mitchell Counties where it was not released. Establishment was recorded in the vicinity of release points in Guthrie, Howard, Plymouth, Shelby, Story, Webster, and Winneshiek Counties. It was not recovered in Fremont from collections taken one year after initial establishment was shown there. Apparently it failed to become established in Harrison County following releases in four consecutive years, but initial establishment was shown in 1950, the fifth year in which it was released.

The history of the fly in study areas in Muscatine County showed that it spread at least nine miles within three years after it was released.

The percentages of borers parasitized by this species in some areas of eastern Iowa in 1948 and 1949, notably in the three counties Cedar, Clinton, and Jones, exceeded that reported in European areas and in the New Jersey area where most of the flies were obtained for release in Iowa.

Macrocentrus gifuensis

Colonization

Adults of this braconid for colonization in Iowa were reared from hibernating European corn borer larvae collected in southern New England, most of them from Taunton, Massachusetts, in 1944, 1945, 1946, and 1947. In the latter year some also came from East Hartford, Connecticut, and after 1947, all came from East Hartford. The history of importations and recoveries of this species in southern New England have been reported elsewhere (10). Apparently the oriental form was the successful one at Taunton and inasmuch as releases in Connecticut were mostly from domestic sources, it is probable that all releases in Iowa were descendents of the oriental form.

This species was available for colonization in greater numbers than all others combined and the 732,139 adults made up nearly 87 per cent of the total parasites released in Iowa. Releases were made in 163 localities in 83 counties as shown in Table 6. In 1944, 1945, and 1946, colonies were released in three localities in each of many eastern coun-

ties, and at five localities in some of them. Releases were made in three successive years in many sites. After 1946, usually only one locality in a county was colonized. The location of all releases are shown in Figure 3. Group releases, i.e., more than one colony in close proximity to one another, were made in Sweetland Township, Muscatine County, in 1948; Harrison Township, Harrison County, Britt, Hancock County, and Liscomb, Marshall County, in 1949, and in the two latter townships in 1950.

Field Status

No collection of host larvae has been taken at the *gifuensis* release

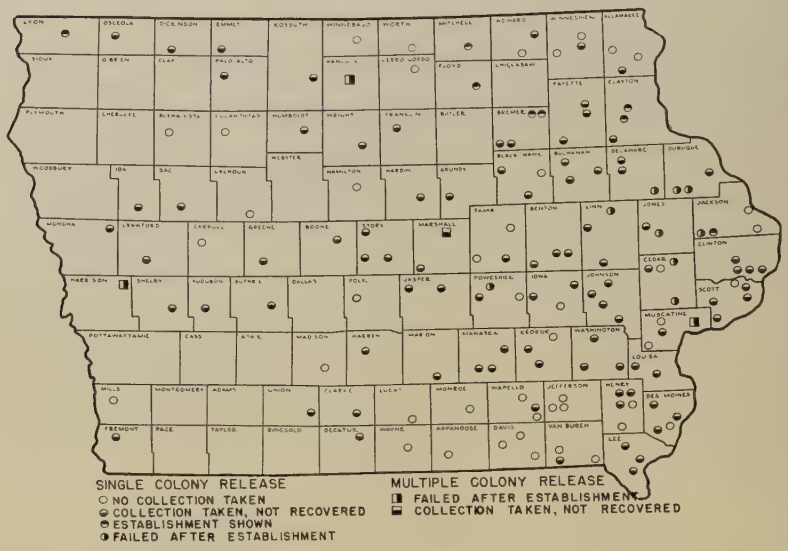

Fig. 3. — Localities in Iowa where *Macrocentrus gifuensis* has been released and the recovery records obtained.

localities in the 16 following counties: Appanoose, Buena Vista, Calhoun, Carroll, Cerro Gordo, Davis, Jefferson, Lucas, Madison, Mills, Monroe, Pocahontas, Van Buren, Wayne, Winnebago, and Worth. *M. gifuensis* was not recovered from collections taken at release localities in 48 counties. It was recovered from summer collections in Bremer County and from collections of hibernating European corn borer larvae in the 15 counties listed in Table 7, but subsequently was not recovered when re-sampling was carried out in the following counties: Black Hawk (1950 summer collections), Cedar (2 localities), Delaware, Dubuque (2 localities), Jackson (2 localities), Jones, Linn, and Poweshiek. The repeated recoveries from Cox Creek and Read Townships, Clayton

TABLE 6

Macrocentrus gifuensis ADULTS RELEASED IN IOWA TO DECEMBER 31, 1951

County	Township	No. released
Year—1944		
Cedar	Dayton	4,046*
"	Pioneer	1,957*
"	Sugar Creek	3,881*
Clinton	Comanche	4,078*
"	Eden	4,023*
"	Orange	4,016*
"	Welton	3,869*
Des Moines	Flint River	3,935*
"	Jackson	3,858
"	Washington	3,871*
Dubuque	Table Mound	1,921*
"	White Water	1,795*
Henry	Canaan	2,024
"	Jackson	1,787*
"	Wayne	1,811
Jackson	Iowa	1,855
"	South Fork	3,513*
"	Tete Des Morts	1,580
"	Washington	1,925*
Johnson	Jefferson	1,907*
"	West Lucas	3,892*
Jones	Cass	3,952*
"	Jackson	3,966
Lee	Jefferson	3,961*
"	Montrose	1,927*
Linn	College	3,932*
"	Jackson	1,694*
Louisa	Columbus City	3,896*
"	Wapello	3,752*
Muscatine	Goshen	3,746*
"	Orono	3,962*
"	Sweetland	5,955*
Scott	Buffalo	3,982*
"	Hickory Grove	4,012*
"	Winfield	6,020
Washington	English River	1,909*
"	Franklin	1,897*
"	Oregon	2,008*
TOTAL		122,115
Year—1945		
Cedar	Dayton	1,800*
"	Pioneer	1,975*
"	Sugar Creek	1,750*
Dubuque	Prairie Creek	1,950*
"	Table Mound	1,825*
"	White Water	1,850*
Henry	Marion	1,700*
Jackson	Monmouth	1,900*
"	South Fork	1,850*
"	Washington	1,900*
Johnson	Jefferson	1,905*
"	Liberty	1,925*
"	Oxford	1,875*
1945 (Cont.)		
Lee	Jefferson	1,825*
"	Marion	1,700*
"	Montrose	1,825*
Linn	College	1,975*
"	Jackson	1,850*
"	Washington	1,900*
Muscatine	Lake	2,202
Scott	Buffalo	1,850*
"	Hickory Grove	1,900*
"	Lincoln	1,850
Tama	Indian Village	1,400*
"	Otter Creek	1,100*
"	Perry	1,020
TOTAL		46,602
Year—1946		
Benton	Eldorado	1,825
"	Fremont	1,790
"	Iowa	1,850
Black Hawk	Barclay	1,928
"	Big Creek	1,772
"	Cedar Falls	1,724
Bremer	Jackson	1,769
"	Jefferson	1,934
"	Sumner	1,832
Buchanan	Fremont	1,796*
"	Hazelton	1,916*
"	Sumner	1,840*
Cedar	Fremont	1,508
"	Pioneer	1,556*
"	Sugar Creek	1,884*
Clayton	Cass	1,858*
"	Cox Creek	1,704*
"	Read	1,928*
Clinton	Comanche	1,527*
"	Orange	1,688*
"	Walton	1,784*
Delaware	Delaware	1,700*
"	Honey Creek	1,740*
"	South Fork	1,788*
Des Moines	Washington	1,880*
Dubuque	Prairie Creek	1,736*
"	White Water	1,775*
Fayette	Jefferson	1,838*
"	Union	1,916*
"	West Field	1,740*
Grundy	Melrose	1,816*
Hardin	Pleasant	1,864*
Henry	Jackson	1,875*
"	Marion	1,680*
"	Scott	1,845*
Iowa	Hartford	1,724*
"	Lenox	1,910*
"	Tray	1,805*

TABLE 6 (Continued)

County	Township	No. released
Year—1946		
Jackson	Monmouth	1,920*
"	South Fork	1,841*
Jasper	Clear Creek	1,475*
"	Malaka	1,856*
Jefferson	Black Hawk	1,880*
"	Center	1,716*
"	Locust Grove	1,724*
Johnson	Jefferson	1,590*
"	Liberty	1,808*
"	Oxford	1,164*
Keokuk	English River	1,912*
"	Sigourney	1,804*
"	Washington	1,884*
Lee	Jefferson	1,775*
"	Marion	1,825*
"	Montrose	1,795*
Linn	College	1,668*
"	Jackson	1,696*
"	Washington	1,804*
Louisa	Columbus City	1,804*
"	Wapello	3,744*
Mahaska	Monroe	1,958
"	Spring Creek	1,945
Marshall	Eden	1,856*
"	Liscomb	1,788*
Muscatine	Goshen	1,826*
"	Orono	1,806*
"	Sweetland	1,910*
Poweshiek	Grant	1,362
"	Sheridan	1,804
"	Warren	1,784
Scott	Butler	1,600*
Story	Lafayette	1,615
"	Nevada	1,640*
"	Washington	1,470*
Tama	Otter Creek	1,582*
Wapello	Highland	1,656
"	Pleasant	1,872
"	Washington	1,650
Washington	English River	1,730*
"	Franklin	1,667*
"	Oregon	1,800*
TOTAL		143,051
Year—1947		
Allamakee	Franklin	1,790
"	Paint Creek	1,900
"	Union Prairie	1,850
Appanoose	Belair	1,925
Audubon	Hamlin	1,853
Boone	Des Moines	1,979
Buchanan	Fremont	3,720*
"	Hazelton	3,640*
"	Sumner	3,820*
Buena Vista	Scott	1,932
Calhoun	Union	1,870
Year—1947		
Carroll	Maple River	1,950
Cerro Gordo	Lime Creek	1,980
Clarke	Osceola	1,905
Clayton	Cass	3,760*
"	Cox Creek	3,875*
"	Read	3,820*
Clinton	Comanche	3,796*
"	Eden	3,929*
"	Orange	3,738*
"	Walton	3,848*
Crawford	Denison	1,973
Davis	Cleveland	1,720
"	Lick Creek	1,750
"	Prairie	1,800
Decatur	Franklin	1,945
Delaware	Delaware	2,900*
"	Honey Creek	3,380*
"	South Fork	3,200*
Des Moines	Benton	3,906
"	Flint River	3,855*
"	Washington	3,955*
Dubuque	Prairie Creek	3,770*
"	Table Mound	1,700*
"	White Water	3,670*
Emmet	Twelve Mile Lake	1,953
Fayette	Jefferson	3,800*
"	Union	5,600*
"	West Field	3,400*
Floyd	Floyd	1,960
Franklin	Marion	1,973
Greene	Jackson	1,969
Grundy	Melrose	1,950*
Guthrie	Valley	1,808
Hamilton	Liberty	1,892
Hancock	Britt	1,975*
Hardin	Pleasant	1,925*
Harrison	Harrison	1,825*
Henry	Jackson	1,730*
"	Marion	1,790*
"	Scott	1,690*
Howard	Paris	1,980
"	Vernon Springs	1,980
Humboldt	Grove	1,925
Ida	Corwin	1,865
Iowa	Hartford	1,925*
"	Lenox	1,930*
"	Tray	1,830*
Jasper	Clear Creek	1,775*
"	Malaka	1,928*
Jefferson	Black Hawk	1,780*
"	Center	1,820*
"	Locust Grove	1,880*
Johnson	Liberty	1,920*
"	Oxford	3,813*
"	West Lucas	3,780*
Jones	Cass	1,911*

1947 (Cont. next page)

TABLE 6 (Continued)

County	Township	No. released
1947 (Cont.)		
Keokuk	English River	1,895*
"	Sigourney	1,765*
"	Washington	1,800*
Kossuth	Wesley	1,983
Lee	Jefferson	1,936*
Loiusa	Columbus City	3,775*
Lucas	Benton	1,955
Madison	Scott	1,985
Mahaska	Lincoln	1,900
Marion	Indiana	1,973
Marshall	Eden	1,907
"	Liscomb	1,960
Mitchell	Burr Oak	1,947
Monona	Cooper	1,953
Monroe	Tray	1,975
Palo Alto	Emmetsburg	1,825
Pocahontas	Sherman	1,968
Sac	Clinton	1,855
Scott	Butler	3,768*
"	Hickory Grove	3,735
Shelby	Center	1,880
Story	Nevada	1,888*
"	Washington	1,925*
Tama	Indian Village	1,913*
Union	Jones	1,987
Van Buren	Des Moines	1,880
"	Farmington	1,870
"	Van Buren	1,760
Wapello	Highland	1,870*
"	Pleasant	1,810*
"	Washington	1,820*
Warren	Lincoln	1,950
Washington	English River	1,896*
"	Franklin	1,915*
"	Oregon	1,590*
Wayne	Union	1,942
Webster	Lost Grove	1,855
Winnebago	Newton	1,975
Winneshiek	Canoe	1,970
"	Decorah	1,935
"	Lincoln	1,970
Worth	Lincoln	1,963
Wright	Lincoln	1,910
TOTAL		257,020

County	Township	No. released
Year—1948		
Dickinson	Okoboji	1,960
Fremont	Green	1,985
Harrison	Harrison	1,267*
Lyon	Rock	1,974
Marshall	Liscomb	3,906*
Mills	Center	1,977
Muscatine	Sweetland	23,135*
Osceola	East Holman	1,984
TOTAL		38,188
Year—1949		
Hancock	Britt	9,372*
Harrison	Harrison	27,847*
Marshall	Liscomb	35,757*
Plymouth	America	1,828
"	Elgin	4,194
"	Grant	1,911
"	Washington	1,912
TOTAL		82,821
Year—1950		
Hancock	Britt	11,121
Marshall	Liscomb	10,054
TOTAL		21,175
Year—1951		
Polk	Saylor	21,167
GRAND TOTAL		732,139

* Released here in more than one year.

County, and Burr Oak Township, Mitchell County, indicate a permanent establishment in these localities. Collections of first brood corn borer larvae from northeastern Bremer County included three parasitized among eight borers from Sumner Township and fourteen parasitized among 247 borers from LeRoy Township. The parasite was released in LeRoy in 1947 and these recoveries indicated establishment and sur-

TABLE 7

THE STATUS OF *Macrocentrus gifuensis* WHEREVER IT HAS BEEN RECOVERED IN IOWA TO THE CLOSE OF 1950

County	Township	Number of borers observed							Percentage of borer parasitized						
		1944	1945	1946	1947	1948	1949	1950	1944	1945	1946	1947	1948	1949	1950
Black Hawk	Cedar Falls	.....	.....	73	.....	.....	84	186[1]	.....	.....	2.7	.....	.....	0	0
Bremer	LeRoy	.....	.....	.....	.....	.....	97	268[1]	.....	.....	.....	.....	.....	0	5.6
"	"	.....	.....	.....	.....	.....	.....	16	.....	.....	.....	.....	.....	.....	0
"	Sumner	.....	.....	.....	.....	.....	.....	8[1]	.....	.....	*	.....	.....	.....	37.5
Cedar	Dayton	96	.....	48	.....	.....	.....	.....	2.1*	*	0	.....	.....	.....	.....
"	Sugar Creek	440	4	42	.....	.....	.....	.....	0.2*	0*	0*	.....	.....	.....	.....
Clayton	Cox Creek	.....	.....	48	48	54	46	.....	.....	.....	0*	4.2*	5.6	21.7	.....
"	Read	.....	.....	48	.....	49	47	.....	.....	.....	0*	*	0	8.5	.....
Delaware	South Fork	.....	.....	88	.....	.....	.....	.....	.....	.....	2.3*	*	.....	.....	.....
Dubuque	Prairie Creek	.....	.....	52	92	.....	.....	.....	.....	*	3.8*	0*	.....	.....	.....
Floyd	Floyd	.....	.....	.....	68	.....	.....	.....	.....	.....	.....	0*	.....	.....	.....
Hancock	Britt	.....	.....	.....	.....	65	247	211	.....	.....	.....	*	3.1	6.1*	0*
Harrison	Harrison	.....	.....	.....	46	175	221	288	.....	.....	.....	0*	0*	0.2	0*
Jackson	Monmouth	.....	.....	44	42	44	.....	.....	.....	*	0*	2.4	0	.....	.....
"	South Fork	.....	.....	524	.....	.....	.....	.....	*	*	0.2	.....	.....	.....	.....
Jones	Jackson	450	487	457	.....	.....	.....	.....	3.3*	0	0	.....	.....	.....	.....
Linn	Jackson	.....	.....	131	.....	.....	45	.....	*	*	2.3*	.....	.....	0	.....
Lyon	Rock	.....	.....	.....	.....	36	51	.....	.....	.....	.....	.....	0*	3.9	.....
Mitchell	Burr Oak	.....	.....	.....	35	.....	189	.....	.....	.....	.....	2.9*	.....	6.9	.....
Muscatine	Sweetland	443	230	258	215	228	210	162	0.2*	0	0.4*	0	1.8*	1.0	0
"	Wilton	261	248	247	222	229	220	208	0	0	0	0	0.9	0.4	0
Poweshiek	Sheridan	.....	.....	27	.....	216	.....	.....	.....	.....	22.2*	.....	0	.....	.....

* Released here this year.

[1] This is a first brood collection, all others were hibernating borers.

vival for three years but no *gifuensis* was recovered from 16 hibernating borers taken from LeRoy in the fall of 1950. The Hancock County recovery in 1948 showed survival for one year and in this locality releases were made again in 1949 and 1950 to test the procedure of continuing releases to insure a population of adults sufficiently high that mating would be accomplished. No *M. gifuensis* was recovered from 211 borers taken from Hancock County in the fall of 1950. In contrast to the procedure in Hancock, releases were discontinued when initial establishment was shown in Harrison and Muscatine. Initial establishment was obtained in Muscatine from 1944, 1946, and 1948 releases, but survival for one year was shown only in 1949 when it was also shown in Lyon County. No *M. gifuensis* was recovered among 1950 fall collections of 284 borers in Harrison County and 452 borers in Muscatine County.

The southernmost recovery of *gifuensis* in the state was in Muscatine County and the only counties where it has persisted or shown evidence of permanent establishment are in the northern half of the state. This species has not demonstrated that it can survive in Iowa.

Horogenes punctorius

Colonization

The biology and seasonal history of *H. punctorius* have been discussed in considerable detail elsewhere (10). Nearly all of the adults for colonization in Iowa were produced from borers collected at East Hartford, Connecticut, although a few of them came from Taunton, Massachusetts, in 1944, 1945, 1946, and 1947, and after 1947 a few came from Burlington, New Jersey.

This parasite was available for colonization in much smaller numbers than either of the two species previously discussed and only 17,116 were released in Iowa. They were more numerous in the earlier years of the program and most were released in eastern counties as shown in Figure 4 and Table 8. Releases were made in 29 townships in 21 counties.

Field Status

Horogenes punctorius was not recovered from collections taken in 15 localities where it was released in Hancock, Harrison, Henry, Iowa, Jackson (two townships), Johnson, Jones, Mahaska, Marshall, Scott (two townships), Story, Tama, and Washington Counties. Apparently it failed to survive after becoming established in Cedar and Clinton Counties as shown in Table 9. The recovery records indicate its permanent establishment in the southwestern part of Benton County where it has persisted for four years and has shown evidence of dispersion and satisfactory percentages of parasitization. Hibernating corn borer larvae, collected from the vicinity of Belle Plaine in the fall of 1950, were dissected in the laboratory and first stage *punctorius* larvae were found in about 10 per cent of them. In other Benton County collections, two *punctorius* were reared from 28 Kane Township

borers and none from 21 borers taken in Iowa Township. Recoveries from Dubuque County give evidence of a permanent establishment there and the recovery from Richland Township, Jones County, in 1948 indicated that dispersion from Dubuque County had occurred but none was found in Richland in 1949. Washington Township, where it was recovered in 1946, adjoins Iowa Township where it was released in Jackson County in 1944, but no later records are available from that vicinity.

No collections to determine the status of the species were taken in

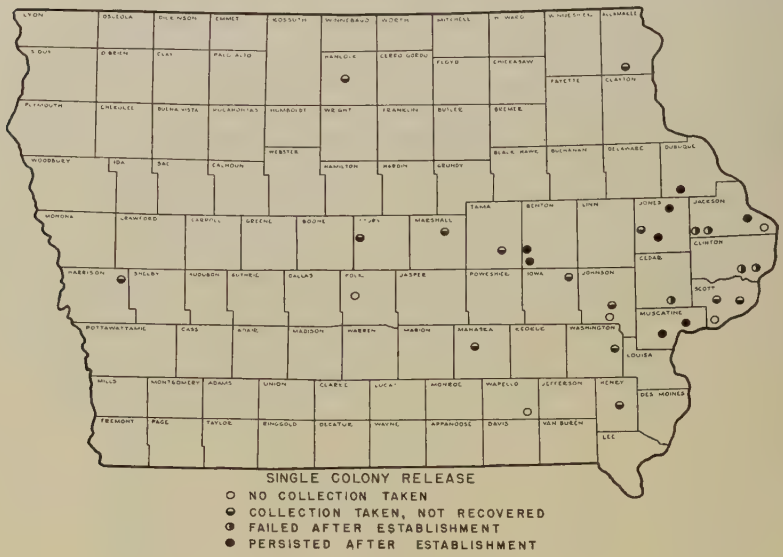

FIG. 4. — Localities in Iowa where *Horogenes punctorius* has been released and the recovery records obtained.

the three release localities in Johnson, Scott, and Wapello Counties. *Horogenes* was released in Sweetland Township in 1944, 1945, and 1946 and in 1945 in Lake Township, Muscatine County. Initial establishment was shown in Sweetland by a recovery in 1945 but none was taken in annual collections in these localities until the fall of 1950 when it was recovered in both of them.

Sympiesis viridula

COLONIZATION

This species was obtained from Canada in 1945, 1946, 1947, and 1948 and was released in one township in each of eight counties as shown in Table 10 and Figure 5. Only one liberation was made at each site, except in Muscatine County where it was released both in 1945 and 1946.

FIELD STATUS

The first recovery of *S. viridula* in Iowa was made in 1948 when it was taken in 16 townships in 10 counties as shown in Table 11. In 1949, it was recovered in four additional townships including two new counties and it was taken in fourteen counties and twenty-five town-

TABLE 8

Horogenes punctorius ADULTS RELEASED IN IOWA TO DECEMBER 31, 1951

County	Township	No. released
Year—1944		
Cedar	Sugar Creek	464*
Clinton	Comanche	458
Dubuque	White Water	283*
Jackson	Iowa	313
Jones	Jackson	380*
Muscatine	Sweetland	371*
Scott	Buffalo	385
"	Hickory Grove	479
TOTAL		3,133
Year—1945		
Cedar	Sugar Creek	481*
Clinton	Eden	497
Henry	Marion	497
Jackson	South Fork	495
Johnson	West Lucas	462
Jones	Jackson	487*
Muscatine	Lake	264
"	Sweetland	499*
Scott	Lincoln	493
Tama	Indian Village	440
Washington	Oregon	495*
TOTAL		5,110
Year—1946		
Benton	Iowa	493
Dubuque	White Water	476
Jackson	Monmouth	481
Johnson	Liberty	482
Jones	Cass	459
Muscatine	Sweetland	480*
Story	Lafayette	196
TOTAL		3,067
Year—1947		
Allamakee	Franklin	470
Harrison	Harrison	497*
Iowa	Lenox	485
Mahaska	Lincoln	475
Wapello	Washington	494
Washington	Oregon	485*
TOTAL		2,906
Year—1948		
Harrison	Harrison	500*
Marshall	Liscomb	356
TOTAL		856
Year—1949		
Hancock	Britt	340
Harrison	Harrison	577*
TOTAL		917
Year—1950		
Harrison	Harrison	485
Year—1951		
Polk	Saylor	642
GRAND TOTAL		17,116

* Released here in more than one year.

ships in 1950. The extensive dispersion and low percentages of parasitization within the relatively short period of time elapsed since it was first released in the state emphasize the observation of Baker, *et al.*, (10) who wrote: "Thus, unlike other corn borer parasites, which build up to considerable concentrations in the immediate vicinity of the release point and spread slowly outward, it has been shown that

TABLE 9

THE STATUS OF *Horogenes punctorius*, WHEREVER IT HAS BEEN RECOVERED IN IOWA TO THE CLOSE OF 1950

County	Township	Number of borers observed							Percentage of borers parasitized						
		1944	1945	1946	1947	1948	1949	1950	1944	1945	1946	1947	1948	1949	1950
Benton	Iowa	.....	.....	12	.....	57	94	21	.....	.....	8.3*	.....	12.3	5.3	0
"	Kane	.....	.....	.....	.....	68	87	28	.....	.....	.....	.....	5.9	8.0	7.1
Cedar	Sugar Creek	440	4	42	.....	.....	.....	.....	0.2*	0*	0	.....	.....	.....	.....
Clinton	Comanche	434	37	47	87	.....	40	.....	0.5*	0	0	1.1	.....	0	.....
"	Eden	.....	33	95	.....	91	.....	.....	.....	3.0*	0	.....	0	.....	.....
Dubuque	White Water	.....	.....	51	110	56	46	.....	*	.....	0*	0.9	3.6	10.9	.....
Jackson	Washington	.....	.....	96	.....	.....	.....	.....	.....	.....	2.1	.....	.....	.....	.....
Jones	Jackson	450	487	457	.....	.....	.....	.....	0.2*	0*	0.2	.....	.....	.....	.....
"	Richland	.....	.....	.....	.....	49	48	.....	.....	.....	.....	.....	4.1	0	.....
Muscatine	Bloomington	229	222	245	227	247	245	208	0	0	0	0	0	0	0.5
"	Sweetland	443	230	258	215	228	210	162	0*	0.4*	0*	0	0	0	1.8

* Released here in more than one year.

Sympiesis viridula is capable of rapidly extending its range without greatly increasing in abundance in any locality." The Boone and Hamilton County recovery points are more than 100 miles from the nearest release points.

The highest percentage of borers observed parasitized by *Sympiesis* in 1948 was 3.5 in Iowa Township, Benton County, and Concord Township, Dubuque County, among 57 borers each. In 1949, the highest parasitization was 2.4 per cent of 42 borers in Prairie Township, Delaware County. Higher percentages were found in the fall of 1950 including 14.3 of 21 borers and 21.4 of 28 borers in Kane and Iowa Townships,

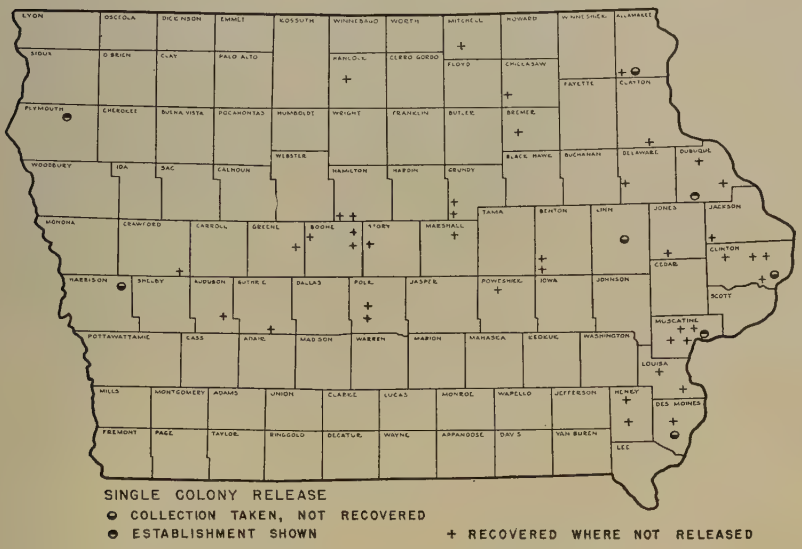

FIG. 5. — Localities in Iowa where *Sympiesis viridula* has been released and where it has been recovered.

Benton County; 5.0 of 20 borers in Warren Township, Bremer County; 13.3 of 165 borers and 8.8 of 57 borers in Felix and Melrose Townships, Grundy County; 6.4 of 211 borers in Britt Township, Hancock County; and 16.0 of 75 borers in Liscomb Township, Marshall County.

Chelonus annulipes

COLONIZATION

Relatively few adults of this species were available from field-collected borers for release in the years 1944 through 1948, but in 1949 and 1950 greater numbers became available through a laboratory breeding program. Sixteen thousand four hundred sixty were released in

eleven localities as shown in Table 12. Grouped releases were made in Harrison County in 1949 and 1950 and in Marshall County in 1950.

Field Status

Chelonus annulipes is not known to be established in Iowa. The only recovery ever made in the state was in Comanche Township, Clinton County, in 1944, but it was not found in collections taken there in each of the next three years and in 1949.

Phaeogenes nigridens

This species was released in Sweetland Township, Muscatine County, in the summer of 1945 when 500 adults were obtained from eastern Massachusetts. Extensive observations on the host in this study area

TABLE 10

**Sympiesis viridula* Adults Released in Iowa to December 31, 1951

County	Township	No. released
Year—1945		
Clinton	Comanche	1,976
Des Moines	Flint River	1,616
Muscatine	Sweetland	1,944*
TOTAL		5,536
Year—1946		
Linn	Marion	3,050
Muscatine	Sweetland	1,934*
TOTAL		4,984
Year—1947		
Allamakee	Franklin	1,990
Dubuque	White Water	1,979
TOTAL		3,969
Year—1948		
Harrison	Harrison	2,000
Plymouth	Elgin	1,997
TOTAL		3,997
GRAND TOTAL		18,486

* Released here in more than one year.

(Area K) showed no *P. nigridens* present, and collections of pupae from overwintered larvae in the spring of 1947, 1948, and 1949 produced none.

Campoplex alkae

Foreign importations of *C. alkae* provided 279 adults for release in Liscomb Township, Marshall County, in 1950 and 719 released in Saylor Township, Polk County, in 1951. This species overwinters in the cocoon stage (4, 36) and no cocoons were found in the fall of 1950.

Microgaster tibialis

Cocoons of *M. tibialis* were imported and provided 33 adults for release in Liscomb Township, Marshall County, in 1950, but no cocoons were found there in the fall of 1950. In 1951, 1,044 adults were released in Saylor Township, Polk County.

TABLE 11

RECOVERIES OF *Sympiesis viridula* IN IOWA TO THE CLOSE OF 1950

County	Township	Number of borers observed:			Percentage of borers parasitized		
		1948	1949	1950	1948	1949	1950
Allamakee	Post	143	32	.....	0	0	[1]
Audubon	Greeley	0	0	5	.....	.....	20.0
Benton	Iowa	57	94	21	3.5	0	14.3
"	Kane	68	87	28	0	0	21.4
Boone	Grant	0	0	11	.....	.....	9.1
"	Jackson	0	12	11	.....	0	9.1
"	Harrison	0	544	218	.....	0	0.4
Bremer	Warren	0	0	20	.....	.....	5.0
Chickasaw	Chickasaw	0	0	.....	.....	.....	[1]
Clayton	Elk	54	48	0	0	2.1	.....
Clinton	Brookfield	64	22	0	3.1	0	.....
"	Deep Creek	49	41	0	2.0	0	.....
"	Eden	91	0	0	1.1	.....	.....
"	Waterford	63	49	0	1.6	0	.....
Crawford	Iowa	0	0	11	.....	.....	9.1
Delaware	Prairie	46	42	0	0	2.4	.....
Des Moines	Franklin	54	46	0	1.9	0	.....
Dubuque	Concord	57	50	0	3.5	0	.....
"	Table Mound	58	52	0	1.7	0	.....
"	White Water	56	46	0	1.8	0	.....
Greene	Junction	0	20	11	.....	0	9.1
Grundy	Felix	0	0	165	.....	.....	13.3
"	Melrose	0	0	57	.....	.....	8.8
Guthrie	Beaver	0	45	3	.....	0	33.3
Hamilton	Clear Lake	0	49	17	.....	0	11.8
"	Marion	0	0	12	.....	.....	8.3
Hancock	Britt	65	247	211	0	0	6.6
Henry	Center	57	47	2	0	2.1	0
"	Wayne	56	52	159	1.8	0	0
Jackson	Monmouth	44	0	0	2.3	.....	.....
Jones	Rome	53	0	0	1.9	.....	.....
Louisa	Concord	50	34	0	2.0	0	.....
"	Jefferson	57	0	0	1.8	.....	.....
Marshall	Liscomb	159	88	75	0	1.1	16.0
Mitchell	Mitchell	0	0	.....	.....	.....	[1]
Muscatine	Bloomington	247	245	208	0	0	2.9
"	Lake	366	398	293	0	0	2.4
"	Sweetland	228	210	162	0	0	3.1
"	Wilton	229	220	208	0	0	4.8
Plymouth	Elgin	105	232	0	1.0	0	.....
Polk	Crocker	0	0	.....	.....	.....	[1]
"	Saylor	0	0	.....	.....	.....	[1]
Poweshiek	Sheridan	216	0	0	0.9	.....	.....
Story	Franklin	0	80	6	.....	0	16.7
"	Lafayette	0	40	1	.....	0	100.0

[1] Taken in the fall, the number of borers observed is not known.

Apanteles thompsoni

Apanteles thompsoni adults were reared at Moorestown in 1951 from imported borers, and 691 adult females were released in Saylor Township, Polk County.

Native Parasites Taken on the European Corn Borer

In studies by the senior author on the native parasites found attacking the European corn borer and the two closely related species, *Pyrausta ainsliei* (Heinrich) and *P. penitalis* (Grote), in Iowa a total of 5,205 borer larvae and pupae were collected, and nearly all reared in the laboratory. The number collected in each county or group of counties is shown in Figure 6. Many additional borers in all stages of development were observed in the field. Although unparasitized individuals were not definitely identified, well over 4,000 were undoubtedly *nubilalis,* and not

TABLE 12

Chelonus annulipes Adults Released in Iowa to December 31, 1951

County	Township	No. released
Year—1944		
Clinton	Comanche	490
Year—1945		
Jones	Jackson	499
Muscatine	Fruitland	499
Scott	Lincoln	496
TOTAL		1,494
Year—1946		
Linn	Marion	265
Muscatine	Sweetland	261
TOTAL		526
Year—1947		
Dubuque	Prairie Creek	490
"	White Water	490
Harrison	Harrison	492*
TOTAL		1,472
Year—1948		
Harrison	Harrison	120*
Marshall	Liscomb	15*
TOTAL		135
Year—1949		
Harrison	Harrison	2,563*
Year—1950		
Harrison	Harrison	4,182*
Marshall	Liscomb	3,289*
TOTAL		7,471
Year—1951		
Polk	Saylor	2,309
GRAND TOTAL		16,460

* Released here in more than one year.

more than 225 were *P. ainsliei* (the smartweed borer). Only a limited number of *P. penitalis* (the lotus borer) were observed.

As shown in Table 13, a total of 230 borer larvae or pupae were found parasitized. Among the six species parasitic on the European corn borer, *Pyraustomyia penitalis* (Coq.) was predominant, but parasitization by this native fly was less than 2.0 per cent of the corn borers observed. *P. penitalis* parasitized a higher percentage of borers than the other native species combined in each of the six years from 1945 through 1950, (Table 14) but only in 1945 with 2.7 per cent, did it exceed 0.5 per cent.

The evidence so far obtained indicates that no native species is adapting itself to sustained attack on the European corn borer, except

possibly *Melanichneuman rubicundus* (Cress.) which has been reared from most of the collections of pupae.

Pyraustomyia penitalis (Coq.)

Allen (1), in describing the habits of this fly as parasitizing the smartweed borer, stated that eggs are laid near the entrance hole of the host on smartweed plants and always on infested nodes. The larvae emerge immediately and wander about, frequently raising their head and waving it about until the entrance hole is found. The tunnel is entered and the host parasitized within a few hours after oviposition.

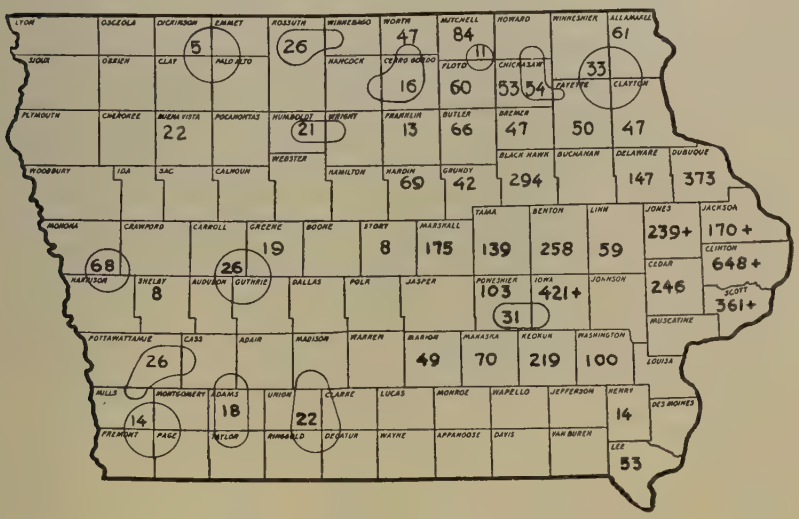

FIG. 6. — Number of borer larvae and pupae collected for observation and rearing of native parasites, 1944–1947.

Ainslie and Cartwright (2) stated that fly and host moth emergence coincided closely in Tennessee, and there were two generations a year.

In the Iowa studies this internal solitary parasite was by far the most common of the native parasites found attacking the European corn borer and the smartweed borer as shown in Table 15. In 1944 in Comanche Township, Clinton County, a collection of 200 larvae from corn yielded 57 parasitized smartweed borers and three parasitized corn borers. Thus the smartweed borers were 28.9 per cent parasitized at the very least. Most of the specimens of this parasite collected elsewhere were from the European corn borer. Although the species is widespread over east-central Iowa, 129 of 197 specimens reared were from Clinton County, due to the fact that most of the smartweed borers were collected there.

Parasite pupation and emergence in the field coincided closely with

that of the European corn borer, as shown in Figure 6, and with that of the smartweed borer. The only rearings of the parasite from summer brood European corn borers were one each from Boone and Henry

TABLE 13

NUMBER OF HOSTS PARASITIZED BY NATIVE PARASITES IN IOWA, 1944–1947[1]

Parasites	Number of hosts parasitized:					Per cent of total native parasites
	P. nubilalis	*P. ainsliei*	*P. penitalis*	*P.* sp.	Total	
Pyraustomyia penitalis	68	112	.....	17	197	85.6
Aplomya caesar	4	1	.....	.....	5	2.2
Microbracon caulicola	2	1	2	1	6	2.6
Bassus agilis	1	1	.....	1	3	1.3
Meteorus loxostege	.....	.....	.....	2	2	0.9
Melanichneumon rubicundus	13	.....	.....	2	15	6.5
Gambrus ultimus	1	.....	.....	1	2	0.9
Cremastus minor (Cush.)	1	.....	.....	.....	.....	.....
TOTAL	90	115	2	24	230	100.0

[1] Excluding cooperative recoveries from *P. nubilalis*.

Counties in the summer of 1950 while all other summer brood rearings were from the smartweed borer.

The average length of the pupal period of 110 individuals was 12 days under laboratory rearing conditions (temperature 80° F.). Variation in the length of the pupal period is shown in Figure 7.

In this study never more than one parasite emerged from a host.

TABLE 14

PARASITIZATION OF HIBERNATING EUROPEAN CORN BORER LARVAE BY NATIVE PARASITES

Year	Number of borers observed	Percentage parasitized by:		
		Pyraustomyia penitalis	Other species	All native parasites
1945	1,851	2.7	0.2	2.9
1946	6,430	0.5	*T*[1]	0.6
1947	2,572	0.3	*T*	0.4
1948	6,384	0.3	*T*	0.4
1949	7,596	0.2	0.1	0.3
1950	9,627	*T*	*T*	0.1

[1] *T* shows that less than 0.1 per cent was parasitized.

The hyperparasite *Eupteromalus viridescens* (Walsh.) was reared from five pupae of *Pyraustomyia penitalis* from *P. ainsliei* taken in Clinton County in 1944. Decker (18), reported rearing *E. cavus* (Ash) (*dubius*) at Ames from this parasite.

During the period from the fall of 1944 through the summer of 1950, *Pyraustomyia penitalis* was reared from the European corn borer from 32 Iowa counties as shown in Table 15 and Figure 8.

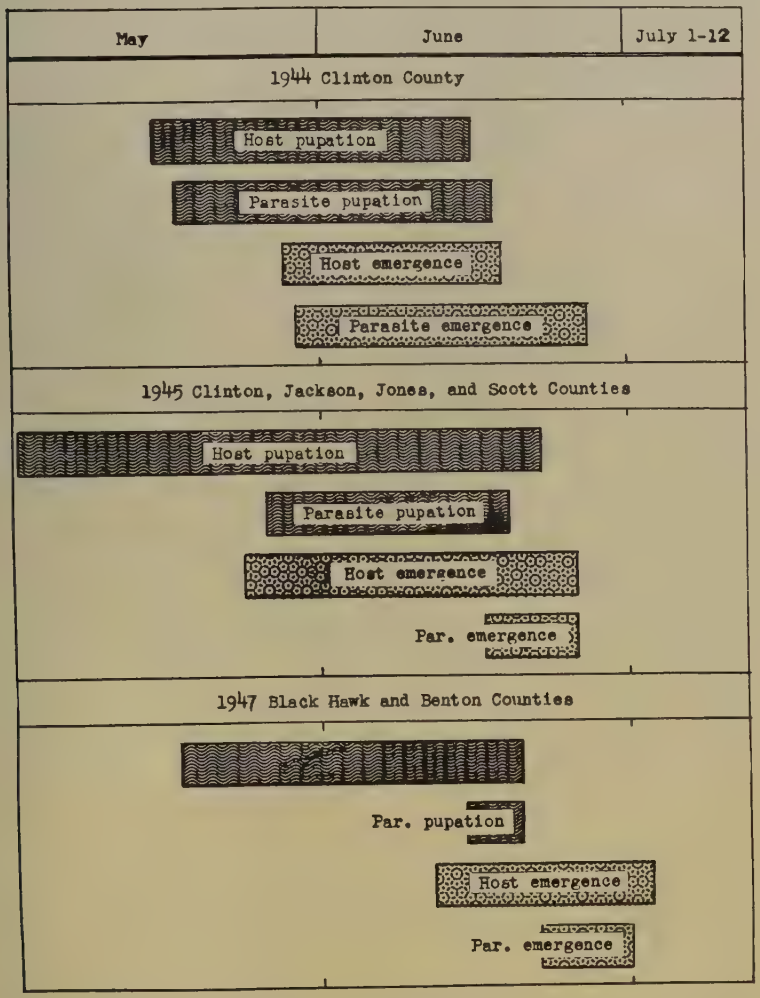

FIG. 7. — Pupation and emergence of the European corn borer and *Pyraustomyia penitalis* in the field (overwintering generation).

Aplomya caesar (Ald.)

Baker, *et al.* (10) report this fly was recovered in greater numbers

from the European corn borer than any other native parasite, except *Trichogramma minutum* Riley. However, in Iowa it was taken less frequently than some other species, and it was recovered from six counties (shown in Figure 9) as follows: Cedar, 1944; Clayton, 1948; Fayette, 1944; Iowa, 1946; Jackson, 1945; Jones, 1944 and 1945. One recovery was made from the smartweed borer in Clinton County in 1944.

Microbracon caulicola Gahan

This braconid species was found only as pupae in clusters near the host remains. No specimens were reared from larvae under labora-

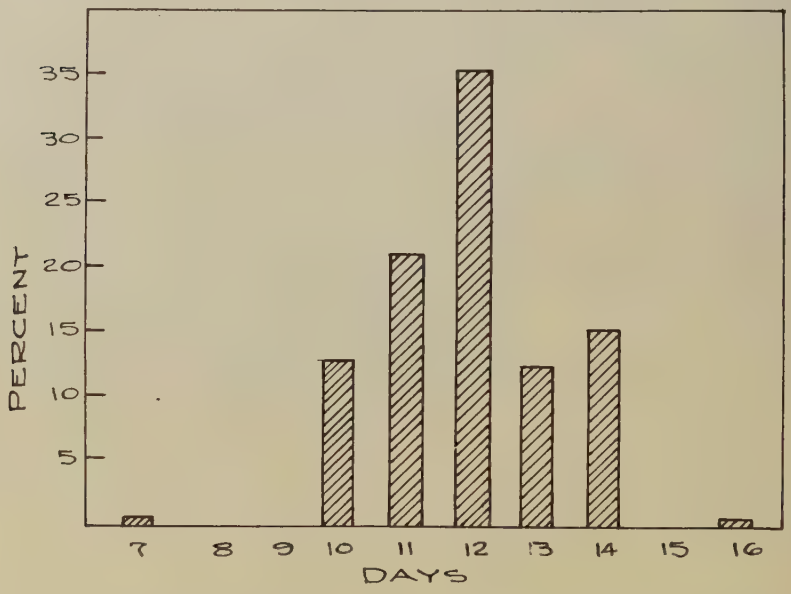

FIG. 8. — Length of the pupal periods of 110 individuals of *Pyraustomyia penitalis* reared under laboratory conditions, winter of 1944–1945.

tory conditions. Six hosts were found parasitized by this species in Clinton and Iowa Counties (Table 16). The average number of individuals per host was 5.8.

There are at least six reports in the literature concerning the presence of this species in Iowa. Ressler (34) referred to the parasitization of fully 50 per cent of the larvae of *Pyrausta ainsliei* which he collected and reared in 1920. He said that each host had four to eight parasite larvae on it. (He placed the parasite in the genus *Aleiodes*). Todd (37) found this species parasitizing *Epiblema otiosana* (Clem.). Drake and Decker (23) wrote that those reared by Todd were collected at

Little Wall Lake (Hamilton County) during the summer of 1926. Hendrickson (32) obtained one adult specimen by sweeping prairie plants at Kelso, July 30, 1928. Decker (18) reported it was one of the most common parasites of the smartweed borer, and that it seldom attacked borers

TABLE 15

Pyraustomyia penitalis Rearings From the European Corn Borer in Iowa

County	Year Recovered	County	Year Recovered
Audubon	1947	Henry	1946, 1949, 1950**
Benton	1944, 1947	Iowa	1946
Black Hawk	1944, 1946	Jackson	1944
Boone	1950*	Johnson	1946
Bremer	1947	Jones	1944, 1945, 1946, 1948
Cedar	1944, 1945	Lee	1944
Clinton	1944, 1945, 1947, 1948	Linn	1944, 1946, 1948
Clayton	1948, 1949	Louisa	1948, 1949
Delaware	1944	Mahaska	1948, 1949
Des Moines	1945	Marshall	1946, 1947, 1948
Dubuque	1944, 1946, 1947, 1948, 1949	Monona	1949
Fayette	1944	Muscatine	1944, 1945, 1946, 1947
Fremont	1949	Poweshiek	1945, 1946, 1948
Grundy	1946	Tama	1945
Hardin	1946	Union	1949
Harrison	1948	Washington	1944, 1946

* Reared from the overwintered and summer brood.
** Reared from the summer brood.

in plants other than in smartweed. However, he found two larvae of *Papaipema nebris* and four larvae of *E. otiosana* parasitized by this species. Baker, *et al.* (10) reported that this species was found attacking European corn borer more often in smartweed than in corn.

The above records indicate that *Microbracon caulicola* is an external, gregarious parasite of host larvae. It overwinters in the pupal stage.

TABLE 16

Collection and Emergence Records of *Microbracon caulicola*

County Collection date of pupae	Date of adult emergence	Number per host	Host
Clinton			
May 29, 1944	June 8 and 9	7	*Pyrausta* sp.
May 30, 1944	June 7	5	" *nubilalis*
"Summer" 1944	.....	1	" *ainsliei*
May 30, 1945	June 12	2	" *nubilalis*
Iowa			
Aug. 28, 1947	Sept. 5	15	" *penitalis*
Aug. 28, 1947	Sept. 5	5	" *penitalis*

Meteorus Loxostege Vier.

One collection of this braconid was made from *Pyrausta sp.* in Lenox Township, Iowa County, where one adult and three pupae were found in borer tunnels in corn on June 11, 1947, and adults emerged from the pupae on June 16. A specimen of *Meteorus,* too malformed for specific identification, was reared from a hibernating European corn borer larva collected from Liscomb Township, Marshall County, in the fall of 1947. No additional recovery was made until 1949 when collections resulted in specimens from Delaware, Dubuque, Hancock, Osceola, Webster, and Woodbury Counties. It was recovered also from spring col-

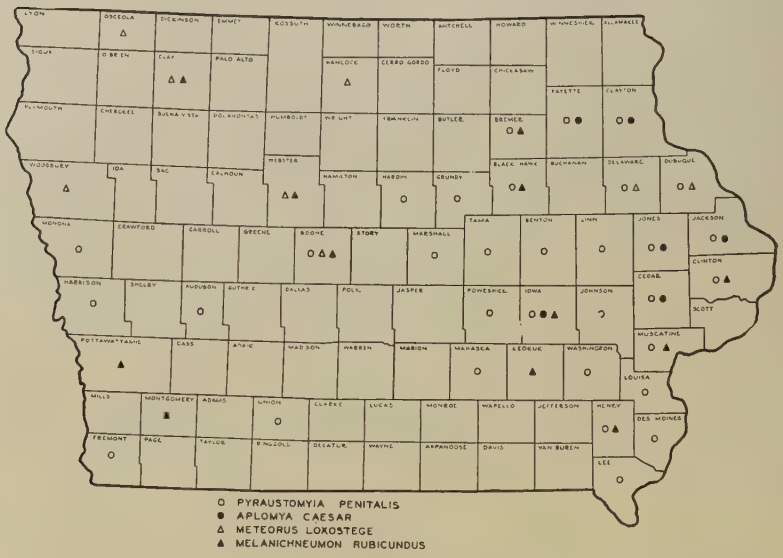

FIG. 9. — The counties in Iowa from which four native species of parasites have been reared from the European corn borer.

lections of the same brood from Boone and Clay Counties and from summer brood collections in 1950 from Bremer and Clay Counties. The county recoveries are shown in Figure 9.

When rearings from European corn borer larvae were made in the laboratory in the spring and summer of 1950 it was noted that the host did not always die immediately after the parasite larvae issued from it but sometimes crawled about for several hours.

Bassus agilis Cress.

Two specimens of this ichneumonid were taken in Clinton County, one as a pupa from *Pyrausta sp.* on June 2, 1944 (emerged on June 5). The other one was collected as a larva from a borer tunnel in a smart-

weed plant on November 4, 1947. It pupated during cold storage and emerged in the laboratory on February 19, 1948.

Recoveries from the European corn borer included one reared from an overwintered larvae from Keokuk County taken on May 16, 1947 (the adult emerged on June 27) and two recoveries from fall collected hibernating larvae, one from Muscatine County, 1945, and one from Keokuk, 1947.

These records show that the species may overwinter either in the host larvae or in the pupal stage.

One adult was taken while sweeping prairie plants in Story County, August 4, 1927 (32).

Gambrus ultimus (Cress.)

Four specimens of this ichneumonid were collected in Clinton County in the field as pupae which had issued from the host. Three taken from June 1 to June 6 on *Pyrausta nubilalis* and *P. sp.*, emerged from June 4 to June 12, 1944. The other one, taken on *P. nubilalis* on June 6, 1945, emerged on June 16.

This species is reported to be a primary parasite on the European corn borer and a hyperparasite on *Horogenes punctorius* in New England (10) Hendrickson (31) reported the collection of an adult (Hoplocryptus *incertulus*) 2.5 miles south of Ames, Story County, on May 12, 1926.

Cremastus minor (Cush.)

This ichneumonid was reared from one of a collection of 48 hibernating European corn borer larvae taken in Orono Township, Muscatine County, in the fall of 1946.

Melanichneumon rubicundus (Cress.)

This ichneumonid was reared only from the European corn borer in these studies. It was recovered from Clinton County from Spring collected pupae of overwintered borers in 1944 when two parasites emerged on June 18 and 22, and in 1945 when five parasites emerged from June 21 to 27. Host pupae collected from Black Hawk, Iowa, and Keokuk Counties from June 13 to 20, 1945, produced six parasites which emerged from June 30 to July 5.

Collections of pupae from overwintered larvae were taken in study area *K*, Muscatine County, in 1947, 1948, and 1949 and the data from them are given in Table 17. *M. rubicundus* was reared from 4.0, 6.7, and 3.1 per cent of the hosts in the three years, respectively.

Pupae were collected from several localities throughout the state in the spring of 1950, and some pupae of the summer brood were collected. Spring collected pupae produced this parasite from six counties and it was recovered in the summer (pupae) from two counties. The counties from which it has been recovered are listed in Table 18, and the distribution in the counties is shown in Figure 9.

Among all recoveries in Iowa, *M. rubicundus* was reared only from

host pupae collected in the field, and the writers believe it is a true pupal parasite and that the adults attack the European corn borer during the latter part of the host's normal pupation period.

Hendrickson (32) collected an adult on May 2, 1928, 2.5 miles north of Ames in Story County.

Eupteromalus tachinae Gahan

Thirty-two specimens of this chalcid were reared from a pupa of

TABLE 17

Melanichneumon rubicundus Reared From Spring Collections and European Corn Borer Pupae Taken in Area *K*, Muscatine County, Iowa

Date collected	Number of host pupae collected			Number of parasites reared		
	1947	1948	1949	1947	1948	1949
May 13			2			
16			5			
20	1		14			
23	4		19			
26			16			
27	4					
29	3					
31			27			2
June 1		18				
2	16					
3		24	26			1
5	23					
7			15			
8		15			4	
10			5			1
11	38	17		2	1	
13	40			2		
15		6				
16	40					
17		12			2	
20	25			1		
22		9				
23	18			2		
25		3				
26	12			2		
TOTAL	224	104	129	9	7	4
Per cent parasitized				4.0	6.7	3.1

an overwintered European corn borer larvae in the spring of 1950. A. B. Gahan who made the determination comments, "I think it probable these (*E. tachinae*) were parasitic on a tachinid in the borer pupae."

Eupteromalus dubius (Ash.)

This chalcid was reared only from *L. stabulans grisescens* parasitic on *Pyrausta nubilalis* and *Pyrausta sp.* Nine rearings were made, six from borers collected in the fall of 1947 in Clayton, Clinton, and Dubuque

Counties, and three from *Lydella* pupae collected November 11, 1947, in Clinton County. The average number of individuals which emerged per host was 7.66.

Decker (17, 18) reported rearing it from the pupae of *Lydella nigrita* (*radicis*) and said that it was present in every large field collection. He also stated, "at Ames, this secondary parasite was reared in large numbers from *Pyraustomyia penitalis*," being, "especially prevalent in the puparia of the overwintering or spring brood."

Eupteromalus viridescens (Walsh)

This chalcid was reared from pupae of *Pyraustomyia penitalis*, the larvae of which had emerged from *Pyrausta ainsliei* but none was taken from *P. penitalis* which had developed on the European corn borer. Emergence from five host pupae collected in Comanche Township,

TABLE 18

Melanichneumon rubicundus Recovery Records From the European Corn Borer in Iowa

County	Year recovered	County	Year recovered
Black Hawk	1945*	Iowa	1945*
Boone	1950†	Keokuk	1945*
Bremer	1950*	Montgomery	1950*
Clay	1950‡	Muscatine	1947*, 1948*, 1949*
Clinton	1944, 1945*	Pottawattamie	1950*
Henry	1950*	Webster	1950*

* Reared from spring collected pupae from overwintered borers.
† Reared from spring collected pupae from overwintered borers and from the summer brood.
‡ Reared from the summer brood.

Clinton County, on June 15, 1944, occurred on June 18, July 2, and July 3. The average number of individuals per host was 17.66.

Decker (18) reported rearing it from overwintering cocoons of *Microbracon caulicola* and *Microplitis gortynae* Riley, and during the summer months from cocoons of *M. gortynae, Macrocentrus pollisteri* Degant, *Apanteles papaipemae* and *M. caulicola,* all of which are primary parasites of lepidopterous stalk borers in Iowa.

SPECIES RECORDED FROM IOWA TAKEN ELSEWHERE ON THE EUROPEAN CORN BORER

Eleven species of parasites reported in the literature as attacking the European corn borer have been taken in Iowa but not in this particular host.

Lixophaga variabilis (Coq.)

Decker (17), in his studies of *Papaipema nebris* (Guen.) reported rearing the larvaevorid *Lixophaga variabilis* and, in 1935, (18) he reported it from three different stalk borers in Iowa. The species has been

reported from plants infested by the European corn borer in Connecticut and New York (10).

Microbracon gelechiae (Ashm.)

This braconid was reared from larvae of *Dioryctria auranticella* Grote taken on Scotch Pine at Ames, Iowa, in 1947 by Farrier. The species has been recorded from the European corn borer in Connecticut, especially when the host borers were found in plants other than corn (10).

Scambus pterophori (Ashm.)

This ichneumonid was reared from *Epiblema otiosana* by Todd (37) and reported in 1927. It also is recorded from the European corn borer in Massachusetts (10).

Itoplectus conquisitor (Say)

Farrier (25) reared this ichneumonid from *Dioryctria auranticella* at Ames, Iowa, in 1947 and it was recorded from Iowa on *Orgyia leucostigma* by Beach (12). Baker, *et al.*, reported it as a primary parasite on the European corn borer and as a secondary parasite, attacking *Horogenes punctorius* in the east (10).

Apanteles pyralidis Mues.

Baker, *et al.*, reported a single colony of this braconid taken on the European corn borer in Indiana. Seven specimens, labeled "Ames, Iowa, Exp. 481; September 19, 1912" are in the Iowa State College collection and specimens from Sioux City, Iowa, are in the U. S. National Museum collection.[5]

Microgaster epagoges Gahan

This braconid is reported (10) on the European corn borer, especially from weeds in the Lake States and New England. The U. S. National Museum contains specimens from Amana, Iowa, and "Iowa Exp. Sta. Pr. No. 30 acc. No. D40 (no locality)."

Gambrus ultimus (Cress.)

Baker, *et al.*, reported this ichneumonid as a primary parasite on the European corn borer and as a secondary parasite attacking *Horogenes punctorius in* New England. It was reported from Iowa by Hendrickson (31) in 1930.

Labrorychus prismaticus (Norton)

Hendrickson (32) in 1931, reported this ichneumonid from Iowa. Baker, *et al.*, reported it from the Lake States and New England on the European corn borer.

[5] C. F. W. Muesebeck, kindly supplied records of certain parasites from the National Museum and the authors gratefully acknowledge his assistance.

Scambus hispae (Harris)

One specimen of this ichneumonid was reported (10) on the European corn borer from Melrose, Mass. The U. S. National Museum has records of it from Ames, Iowa.

Enicospilus purgator (Say)

One specimen of this ichneumonid is reported from the European corn borer, taken in Ohio, and the U. S. National Museum has records of it from Sioux City and Johnson County, Iowa.

Melanichneumon brevicinctor (Say)

This ichneumonid is reported (10) attacking the European corn borer in New England. Three specimens in the collection at Iowa State College are labeled, "Ames, Iowa, Exp. Sta., May 7, 1894, Ames, Iowa," and one has no label. U. S. National Museum records are from Sioux City and Van Buren County, Iowa.

Dibrachys cavus (Walk.)

This chalcid is more often taken as a hyperparasite (10), but it has been taken as a primary parasite on the Euorpean corn borer. Three specimens, taken by Farrier (25) are in the Iowa State College collection, two are labeled July 3, 1947, and one July 18, 1947. U. S. National Museum records are from Sioux City and Van Buren County, Iowa.

Trichogramma minutum Riley

Baker, *et al.*, discuss the roll of this chalcid as a European corn borer parasite in considerable detail. The U. S. National Museum contains specimens from Ames, Iowa.

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COMPARATIVE DEVELOPMENT OF THE EMBRYOS OF INBRED AND HYBRID MAIZE[1]

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Morphological expressions of heterosis may be sought at various points in the diploid phase of the life cycle of a seed plant. Considerable attention has been given, by previous workers, to the morphological expression of hybrid vigor in maize, from the time of seed germination to maturity of the plant.

Comparative increase in dry weight has been used as the basis for comparison in most investigations of heterosis, as expressed during seed development. Statistical analysis of dry weights of maize embryos at various intervals during development have shown differences in the relative growth rates of inbred and hybrid embryos. Because of difficulties encountered in making mass dissections, these investigations have not taken into consideration possible differences in the expression of heterosis in different parts of the embryonic axis. The present investigation was undertaken to explore the possibility that heterosis may be expressed in the histological and morphological development of different parts of the maize embryo.

REVIEW OF LITERATURE

The literature pertaining to the comparative development of inbred and hybrid maize embryos may be divided into two categories: (1) studies which have used weight as the basis of comparison; (2) studies which have used cytological, histological, or morphological observations as a basis for comparison between inbreds and hybrids.

East (6) reported that hybrid maize embryos did not always exceed the parent inbreds in size, whereas Murdoch (11) found that mature hybrid embryos had greater dry weight than either parent. Shafer (14) compared final dry weights of the embryonic axes of inbred and hybrid maize. In some hybrids the embryo axes were no heavier than those of the parent inbreds, whereas in other hybrids, the hybrid axes exceeded the parents in weight.

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Ashby (1) also compared weights of the embryonic axes of inbred and hybrid maize, and found significant differences between the inbreds and the hybrids, and between the reciprocals. These differences between the reciprocals were attributed to maternal influence. He suggested that rate of growth was determined by the more vigorous parent and that this difference in rate of growth was of prime importance in the final development of the mature plant. This hypothesis stimulated additional investigations.

Sprague (15) showed that hybrid embryos may be smaller than, equal to, or larger than the maternal parent. He pointed out that since the hybrid and inbred zygotes are probably of practically identical weight, differences in mature weight indicate differences in growth rate, and suggested that the growth of the hybrid is determined by the complimentary or additive effect of genes from both parents.

Paddick and Sprague (12) compared the dry weights of hybrid and inbred embryos grown on the same ear. Both parents appeared to exert some influence on embryo weight. Germ size varied greatly in reciprocal hybrid kernels.

Bernstein (4) found that the size of hybrid embryos of maize exceeded inbred embryos during the first three weeks after pollination. In later stages of embryo development the hybrid embryos were not consistently heavier than the inbreds when compared on the basis of dry weight.

Kempton and McLane (9) reported that hybrid embryos exceeded inbred embryos in rate of increase in dry weight during early development. These differences did not remain constant throughout embryonic growth.

Groszmann and Sprague (8) compared dry weights of the embryos in pop and dent inbreds and their reciprocal hybrids. Differences between the dent inbred and the dent x pop hybrid were not significant. The dent x pop hybrid embryo was significantly heavier than the pop inbred. The rate of increase of dry weight of the embryo in the dent inbred and in the dent x pop hybrid were not significant. The pop x dent hybrid exceeded the inbred pop in rate of increase in embryo weight.

Investigations utilizing cytological, histological, or morphological criteria are not numerous. Bindloss (5) compared the plumular meristems and nuclear volumes in mature inbred and hybrid maize embryos. The lines used were the same as those used by Ashby (1). Hybrid vigor, as expressed in embryo weight and in post-germination growth rate, had been reported for these lines. There was no correlation between embryo weight and size of plumular meristems. The size of the meristem in the hybrid appeared to be no greater than that of the larger parent. Positive correlation between nuclear size and heterosis was evident in only one of the three pairs of reciprocal hybrids.

Magee (10) compared organ initiation in embryos of inbred pop and dent maize and the reciprocal hybrids. The time of leaf initiation was used as a criterion for comparing plumule development. Both reciprocals exhibited heterosis with respect to the time of leaf initiation. Radicle

development was compared on the basis of the formation of the cleft between the radicle and coleorhiza, and the enlargement of the metaxylem elements. The reciprocal hybrids surpassed both inbred parents in the radicle development. Procambial strands in the scutellum differentiated earlier in the hybrids than in their female parents. The hybrid embryos resembled their female parents in shape.

MATERIAL AND METHODS

The inbred lines of maize used in these studies were furnished by Dr. G. F. Sprague. Preliminary observations were made on a considerable number of inbred lines (7), two of which seemed to be obviously different in the rate of embryo development. These two inbreds, L-317 and Bl-349, and their reciprocal hybrids, were used for the detailed study.

A split-ear pollination technique was devised, so that both sibbed and crossed kernels were on the same ear. A median longitudinal split was made at the tip of each ear at the time the silks were cut back, approximately twenty-four hours prior to pollination. Two glassine ear shoot bags were fastened back to back with a strip of manila paper which was rigid enough to be slipped into the split ear. When the silks were grown out, one-half of the ear was sibbed and the other half crossed. Three ears from each inbred, and from each hybrid, were collected two days after pollination and at five day intervals thereafter, from 5 to 40 days after pollination.

A sample of kernels was removed from just below the center of each half of each ear. The reliability of this method of sampling has been confirmed by Bell (3). The 2- to 15-day kernels were trimmed and killed whole. In later stages only dissected embryos were killed. The dehydration and embedding schedules used were those described by Sass (13). Whole kernels of the younger stages were cut in longitudinal section and transverse section 10 to 15 microns in thickness. Individual embryos of the older stages were cut at 10 microns. The haemalum-safranin staining schedule was used (13).

Serial transverse sections made it possible to examine all levels of the embryo critically. Four regions of the older kernels were chosen for extensive comparisons. These were: (1) the apical meristem at the level of initiation of the youngest leaf; (2) the first internode above the scutellum; (3) the region of seminal root initiation; (4) the radicle at the most proximal region at which it is separated from the coleorhiza by a cleft.

Transverse sections of the plumule at the level of the apical meristem were projected and drawn. A magnification of 210x was used for 20-day embryos, and 100x for the older stages. The transverse sectional area of the coleoptile, of each leaf, and of the apical meristem was measured with a planimeter and tabulated in terms of square millimeters of actual area. The transverse area of the cortex and stele of the first internode was determined for each line at 30, 35, and 40 days.

The radicle was measured at the most proximal level at which it is

separated from the coleorhiza by a cleft. Measurements were made of the area of the stele and cortex, and the total transverse area of metaxylem elements was determined by averaging 10 measurements.

At the level of seminal root initiation, it was found impractical to make comparisons on the basis of area measurements, because the seminal roots arise at an angle to the main axis, and at different levels. The lines were compared on the basis of the relative time of seminal root initiation and differentiation.

OBSERVATIONS

Microscopic examinations of inbred and hybrid embryos of different ages were made, and transverse area measurements were made of embryonic organs of the inbreds L-317 and Bl-349, and their reciprocal hybrids. In the analysis of data, it was desired to make comparisons: (1) between inbreds; (2) between hybrids; (3) between inbred L-317 and hybrid L-317 x Bl-349; (4) between inbred Bl-349 and hybrid Bl-349 x L-317. The first two comparisons were accomplished by analysis of variance. The remaining orthoganal degree of freedom contains no information on either of the remaining two comparisons. Therefore, the least significant difference was used to compare the hybrids and their maternal parents. Repeated application of the least significant difference to a set of means, alters the probabilities involved, so that only approximate tests of significance may be obtained. These approximate tests for significant differences in size were used in conjunction with morphological and histological comparisons.

EARLY EMBRYONIC DEVELOPMENT

Two days after pollination the inbreds L-317 and Bl-349, and their two reciprocal hybrids, showed no obvious differences in development. Four to eight nuclei were observed in the embryo sacs and no division of the zygote was noted. Five days after pollination, differences in embryo development, size and shape were evident. Actual differences in embryo size at this age cannot be measured readily, but the embryo of L-317 was found to be obviously smaller than that of Bl-349. The embryos of the reciprocal hybrids were intermediate in size.

Embryo shape differs in lines of maize. The embryos of Bl-349 and of both reciprocal hybrids are somewhat flattened on the anterior face, and at five days show evidence of increased meristematic activity in the region of plumule initiation. The 10- and 15-day embryos of Bl-349 were found to be more advanced in plumule development than those of L-317. No obvious differences in radicle development were observed. The hybrid embryos were intermediate in size.

DEVELOPMENT OF THE PLUMULE

The region chosen for the comparison of plumule development in inbreds and hybrids, was the transverse section at the level of the apical meristem (Fig. 1). In transverse aspect at this level, the embryos of the inbreds L-317 and Bl-349 are quite different in shape. The plumule of

L-317 is elliptical in transverse section, except for a slight flattening on the anterior face (Fig. 2). The plumule of Bl-349 is elongated in a plane perpendicular to the anterior face, and somewhat tapered on the anterior and posterior edges (Fig. 3). The embryos of the reciprocal hybrids are intermediate in shape, but tend to resemble those of the female parent (Figs. 3, 4).

The inbreds exhibit differences in the histological development of

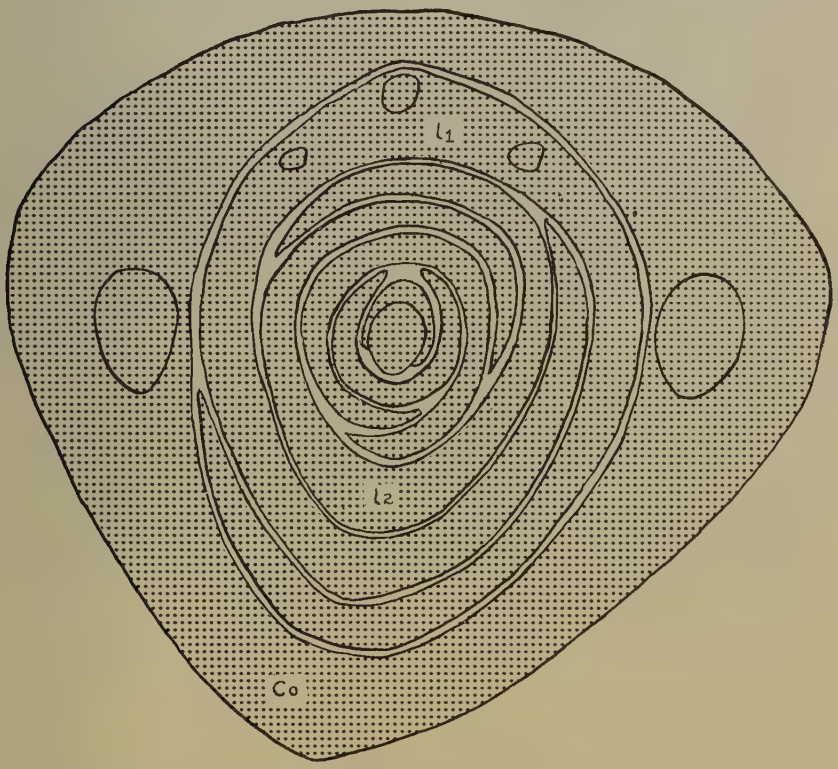

FIG. 1. Semidiagrammatic drawing of a transverse section of a morphologically mature maize embryo showing Co, Coleoptile; L1, first leaf; L2, second leaf, and arrangement of other three leaves.

the plumule. Twenty days after pollination the marginal leaf meristems of Bl-349 exceed those of L-317 in activity. The extent of activity in these meristems cannot be compared readily by measurement, but differences may be judged by the extent of overlapping of the margins of the older leaves, and by the visual evidence of marginal expansion in the younger leaves (Figs. 2, 3). The inbred Bl-349 exceeds L-317 with respect to lateral expansion of leaf No. 1 and leaf No. 2. The hybrids appear to be

intermediate, and resemble their female parents with respect to the relative activity of marginal leaf meristems (Figs. 4, 5).

Differences were also observed between lines in the rate of leaf initiation. Twenty days after pollination the third leaf of L-317 consists of a ridge of cells, two to three cells thick along the anterior face (Fig. 2). The third leaf of Bl-349 had already undergone considerable cell division and had become a well-defined leaf, four to five cells thick (Fig. 3). The hybrids appear to be intermediate in the rate of leaf initiation.

Comparisons between the inbreds, and between the reciprocal hybrids, on the basis of area measurements of plumular organs, are presented in Table 1.

Differences between the two inbred lines are significant, whereas differences between the two reciprocal hybrids are non-significant.

TABLE 1

ANALYSIS OF VARIANCE OF TRANSVERSE SECTIONAL AREAS OF PLUMULAR ORGANS, 20 DAYS AFTER POLLINATION

	M.S. Between Groups (3 d.f.)	M.S. Within Groups (36 d.f.)	M.S. for Testing the Difference Between	
			Inbreds (1 d.f.)	Hybrids (1 d.f.)
Coleoptile	5027*	139	14151†	312
1st leaf	1309	42	3564†	92
2nd leaf	220	7	594†	5
3rd leaf	19	1	54†	1
Apical meristem	33	1	97†	2

* All entries x 10^{-6}
† F larger than tabulated 1% value

Table 2 contains measurements of mean transverse areas of plumular organs of 10 different embryos, of each inbred and of each hybrid 20 days after pollination. The mean area of all organs, except leaf 3, is significantly larger in the hybrid L-317 x Bl-349, than in the inbred L-317. The mean area for all organs is significantly larger in Bl-349 than in the hybrid Bl-349 x L-317.

Thirty days after pollination, all but one of the embryos of L-317 that were examined had initiated the fourth leaf (Fig. 6). The inbred Bl-349 has a well-defined fourth leaf with provascular strands (Fig. 7). In the hybrid L-317 x Bl-349, the fourth leaf arises earlier than in the inbred L-317, but not as early as in the male parent (Fig. 8). The hybrid Bl-349 x L-317 has a well-defined fourth leaf with evident provascular strands (Fig. 9). The activity of marginal leaf meristems of Bl-349 exceeds that of L-317, the hybrid L-317 x Bl-349 is intermediate and resembles its

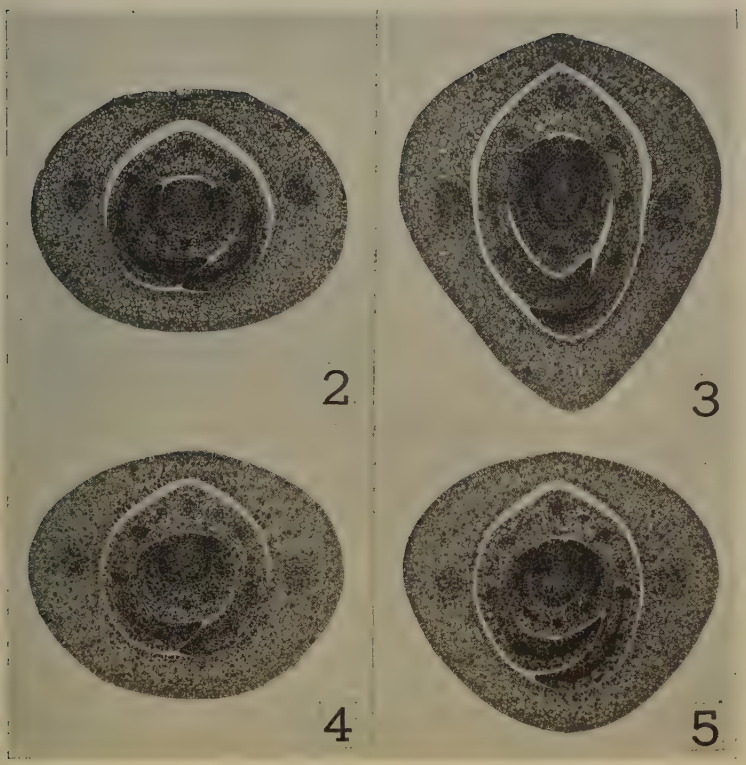

FIG.

2. Transverse section of the plumule of inbred L-317 showing the coleoptile, two leaves, and the minute primordium of the third leaf, 20 days. x58
3. Transverse section of plumule of inbred Bl-349 showing the coleoptile and three leaves, 20 days. x58
4. Transverse section of the plumule of hybrid L-317 x Bl-349, 20 days. x58
5. Transverse section of the plumule of hybrid Bl-349 x L-317, 20 days. x58

TABLE 2

MEAN TRANSVERSE SECTIONAL AREA OF PLUMULAR ORGANS, 20 DAYS AFTER POLLINATION (in sq. mm.)

	L-317	L-317 x Bl-349	Bl-349 x L-317	Bl-349	L.S.D.
Coleoptile	.1353	.1501	.1580	.1885	.0143
1st leaf	.0551	.0611	.0654	.0818	.0025
2nd leaf	.0180	.0215	.0205	.0289	.0032
3rd leaf	.0023	.0032	.0037	.0056	.0012
Apical meristem	.0058	.0075	.0082	.0102	.0012

female parent, and the reciprocal hybrid is not obviously different from Bl-349.

Comparisons between inbreds, and between hybrids at 30 days are presented in Table 3. The differences between inbreds are significant for all measurements. The hybrids are significantly different, except for the areas of respective apical meristems.

TABLE 3

ANALYSIS OF VARIANCE OF TRANSVERSE SECTIONAL AREA OF PLUMULAR ORGANS, 30 DAYS AFTER POLLINATION

	M.S. Between Groups (3 d.f.)	M.S. Within Groups		M.S. for Testing the Difference Between	
			(d.f.)	Inbreds (1 d.f.)	Hybrids (1 d.f.)
Coleoptile	37281 *	1450	36	93708‡	9724†
1st leaf	8829	292	36	18911‡	6266‡
2nd leaf	2029	85	36	4263‡	911‡
3rd leaf	271	13	36	720‡	68†
4th leaf	107	3	35	275‡	16†
Apical meristem	21	1	36	61‡	2

* All entries x 10^{-6}
† F larger than tabulated 5% value
‡ F larger than tabulated 1% value

Mean transverse sectional areas of plumular organs and least significant differences are given in Table 4. Mean values for the hybrid L-317 x Bl-349 are significantly larger than those of the inbred L-317. The differences between Bl-349 x L-317 and Bl-349 are non-significant.

Thirty-five days after pollination the embryos of L-317 vary in the

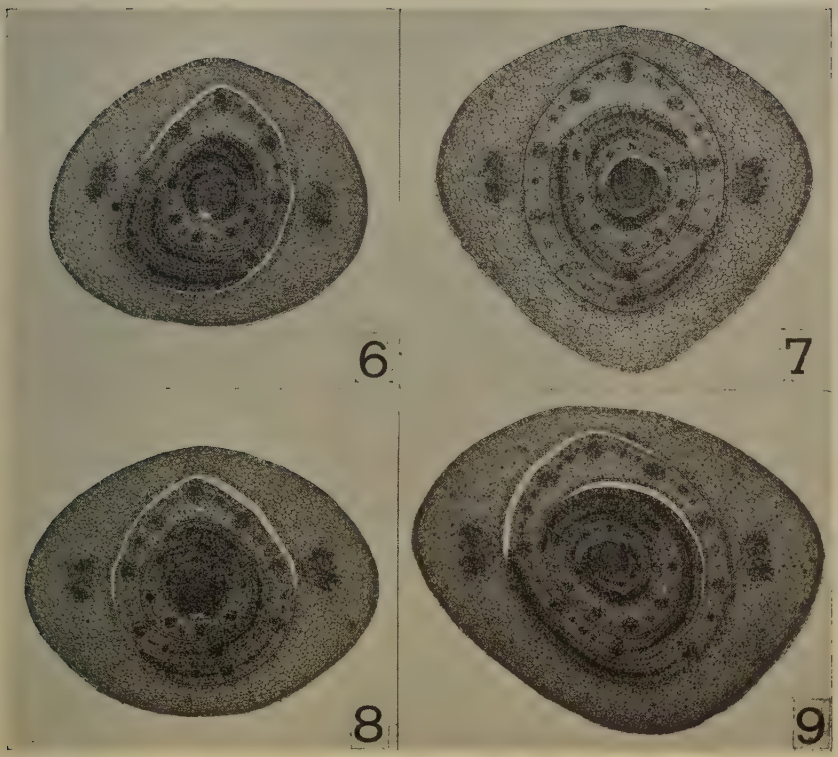

FIG.

6. Transverse section of the plumule of inbred L-317 showing three leaves, 30 days. x39
7. Transverse section of the plumule of the inbred Bl-349 showing four leaves, 30 days. x39
8. Transverse section of the plumule of the hybrid L-317 x Bl-349 showing four leaves, 30 days. x39
9. Transverse section of the plumule of the hybrid Bl-349 x L-317 showing four leaves, 30 days. x39

TABLE 4

MEAN TRANSVERSE SECTIONAL AREA OF PLUMULAR ORGANS, 30 DAYS AFTER POLLINATION (in sq. mm.)

	L-317	L-317 x Bl-349	Bl-349 x L-317	Bl-349	L.S.D.
Coleoptile	.3294	.4048	.4489	.4663	.0463
1st leaf	.1458	.1703	.2057	.2073	.0208
2nd leaf	.0506	.0680	.0815	.0798	.0112
3rd leaf	.0172	.0229	.0266	.0292	.0044
4th leaf	.0034	.0086	.0104	.0114	.0022
Apical meristem	.0059	.0076	.0082	.0094	.0012

number of leaves present. Three of the ten embryos examined had not initiated the fourth leaf. The remaining seven embryos had formed the fourth leaf primordium. The embryo of inbred Bl-349 consistently has five leaves, and Bl-349 x L-317 has five leaves. The relative activity of marginal leaf meristems is greater in L-317 x Bl-349 than in the female parent, and there is no obvious difference between the inbred Bl-349 and the hybrid Bl-349 x L-317.

Comparisons of plumular organs of inbreds and hybrids are presented in Table 5.

The differences between inbreds are significant. The differences be-

TABLE 5

ANALYSIS OF VARIANCE OF TRANSVERSE SECTIONAL AREAS OF PLUMULAR ORGANS, 35 DAYS AFTER POLLINATION

	M.S. Between Groups (3 d.f.)	M.S. Within Groups		M.S. for Testing the Difference Between	
			(d.f.)	Inbreds (1 d.f.)	Hybrids (1 d.f.)
Coleoptile	31783*	1383	36	81409‡	5645†
1st leaf	10310	681	36	24221‡	4651†
2nd leaf	2899	191	36	5445‡	2668‡
3rd leaf	769	43	36	1620‡	616‡
4th leaf	86	13	33	207‡	5
5th leaf	9	1	24	.....	7†
Apical meristem	7	3	36	13†	8

* All entries x 10^{-6}
† F larger than tabulated 5% value
‡ F larger than tabulated 1% value

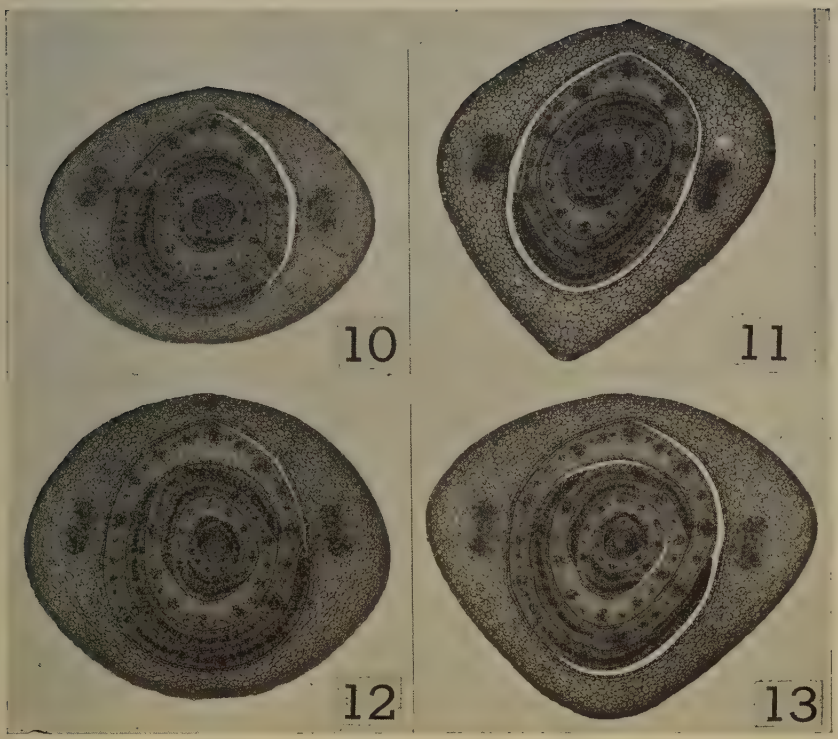

Fig.

10. Transverse section of the plumule of the inbred L-317 showing four leaves, and the minute primordium of the fifth leaf, 40 days. x33
11. Transverse section of the plumule of the inbred Bl-349 showing five leaves, 40 days. x33
12. Transverse section of the plumule of the hybrid L-317 x Bl-349 showing five leaves, 40 days. x33
13. Transverse section of the plumule of the hybrid Bl-349 x L-317 showing five leaves, 40 days. x33

tween hybrids are also significant, except for the area of the fourth leaf and of the apical meristem.

Mean transverse sectional area of plumular organs and least significant differences are given in Table 6. Transverse sectional area in plumular organs is larger in L-317 x Bl-349 than in the female parent. The differences do not appear to be significant for areas of the second and third leaves, and for the areas of apical meristems. The differences between Bl-349 x L-317 and its female parent are non-significant.

Forty days after pollination the inbred L-317 had initiated the fifth leaf in only three of the ten embryos examined (Fig. 10). At this age, the inbred Bl-349 and both reciprocal hybrids have well-defined fifth leaves

TABLE 6

MEAN TRANSVERSE SECTIONAL AREAS OF PLUMULAR ORGANS, 35 DAYS AFTER POLLINATION (in sq. mm.)

	L-317	L-317 x Bl-349	Bl-349 x L-317	Bl-349	L.S.D.
Coleoptile	.3943	.4701	.5037	.5219	.0452
1st leaf	.1660	.1999	.2304	.2356	.0317
2nd leaf	.0709	.0835	.1066	.1039	.0168
3rd leaf	.0228	.0289	.0400	.0408	.0080
4th leaf	.0061	.0146	.0156	.0158	.0048
5th leaf		.0023	.0042	.0038	.0013
Apical meristem	.0066	.0065	.0078	.0082	.0021

(Figs. 11, 12, and 13). The hybrid L-317 x Bl-349 exceeds the female parent and equals its male parent in regard to activity of marginal leaf meristems, as judged by visual evidence of lateral expansion. The hybrid Bl-349 x L-317 exceeds both parents in activity of marginal leaf meristems.

Comparisons of plumular organs of inbreds and hybrids are presented in Table 7.

The differences between inbreds are significant for all plumular organs. The hybrids also differ significantly, except in the area of apical meristems.

Mean transverse sectional area of plumular organs and the leaf significant differences are presented in Table 8.

The hybrid L-317 x Bl-349 is significantly larger than its female parent. The hybrid Bl-349 x L-317 is not significantly different from its female parent except in transverse sectional area of the coleoptile and the first and fourth leaves.

DEVELOPMENT OF THE FIRST INTERNODE

The first internode of the maize embryo is that portion of the embryonic axis which lies bewteen the scutellar plate and the coleoptile node.

TABLE 7

ANALYSIS OF VARIANCE OF TRANSVERSE SECTIONAL AREAS OF PLUMULAR ORGANS, 40 DAYS AFTER POLLINATION

	M.S. Between Groups (3 d.f.)	M. S.Within Groups		M.S. for Testing the Difference Between	
			(d.f.)	Inbreds (1 d.f.)	Hybrids (1 d.f.)
Coleoptile	65015*	1383	36	84630‡	60500‡
1st leaf	14634	455	36	13781‡	11713‡
2nd leaf	4435	319	36	4500‡	2531‡
3rd leaf	983	36	36	1549‡	451‡
4th leaf	199	9	36	218‡	168‡
5th leaf	18	1	29	18‡	36‡
Apical meristem	3	1	36	6†	2

* x 10^{-6}
† F larger than tabulated 5% value
‡ F larger than tabulated 1% value

As the embryo approaches maturity there is considerable growth in this region, primarily as a result of cell enlargement. Transverse sectional area measurements of cortex and stele were taken at comparable levels near the basal portion of this internode, above the region of seminal root initiation. Comparisons were limited to embryos collected 30, 35, and 40 days after pollination. Since there are no obvious histological differences between the inbreds and hybrids at this particular level, comparisons are based on transverse sectional areas. Table 9 presents comparisons between inbreds and between hybrids 30, 35, and 40 days after pollination.

The inbred Bl-349 is significantly larger than L-317 in transverse

TABLE 8

MEAN TRANSVERSE SECTIONAL AREA OF PLUMULAR ORGANS, 40 DAYS AFTER POLLINATION (in sq. mm.)

	L-317	L-317 x Bl-349	Bl-349 x L-317	Bl-349	L.S.D.
Coleoptile	.4369	.5176	.6276	.5670	.0452
1st leaf	.1899	.2339	.2823	.2424	.0259
2nd leaf	.0814	.1102	.1327	.1114	.0217
3rd leaf	.0312	.0450	.0545	.0488	.0073
4th leaf	.0118	.0168	.0226	.0184	.0036
5th leaf	.0007	.0033	.0060	.0052	.0018
Apical meristem	.0075	.0080	.0086	.0086	.0012

sectional area of the stele and cortex 30, 35, and 40 days after pollination. The hybrid Bl-349 x L-317 is significantly larger than its reciprocal at 30, 35, and 40 days.

Mean transverse sectional areas for each inbred and each hybrid, and the least significant differences appear in Table 10.

TABLE 9

ANALYSIS OF VARIANCE OF TRANSVERSE SECTIONAL AREAS OF THE FIRST INTERNODES, 30, 35, AND 40 DAYS AFTER POLLINATION

	M.S. Between Groups (3 d.f.)	M.S. Within Groups (36 d.f.)	M.S. for Testing the Difference Between	
			Inbreds (1 d.f.)	Hybrids (1 d.f.)
30 Days				
Cortex	230353*	2766	646561‡	44274‡
Stele	6037	264	14851‡	1748†
Total	307973	3881	857394‡	63619‡
35 Days				
Cortex	236058	3850	680805‡	20608†
Stele	14731	391	30264‡	13520‡
Total	358720	6006	998151‡	67512‡
40 Days				
Cortex	383548	5716	912926‡	199400‡
Stele	16209	415	27306‡	10397‡
Total	549008	8288	1256007‡	300860‡

* All entries $\times 10^{-6}$
† F larger than tabulated 5% value
‡ F larger than tabulated 1% value

The hybrid L-317 x Bl-349 is significantly larger than its female parent in the transverse sectional area of the stele, except that at 35 days the difference between mean cortex areas appears to be non-significant. The inbred Bl-349 is significantly larger than the hybrid Bl-349 x L-317 in transverse area of the cortex, 30 and 35 days after pollination. Forty days after pollination the difference between mean cortex areas is non-significant. The hybrid Bl-349 x L-317 and its maternal parent do not differ significantly in transverse area of the stele.

DEVELOPMENT OF THE RADICLE

The radicles of the inbreds L-317 and Bl-349, and their reciprocal hybrids show obvious differences in shape and dimensions of transverse

TABLE 10
MEAN TRANSVERSE SECTIONAL AREA OF 1ST INTERNODE, 30, 35, AND 40 DAYS AFTER POLLINATION
(in sq. mm.)

	L-317	L-317 x Bl-349	Bl-349 x L-317	Bl-349	L.S.D.
30 Days					
Cortex	.4792	.6167	.7108	.8388	.0640
Stele	.1063	.1365	.1552	.1608	.0198
Total	.5855	.7532	.8660	.9996	.0758
35 Days					
Cortex	.5709	.7493	.8135	.9399	.0754
Stele	.1283	.1476	.1996	.2061	.0240
Total	.6992	.8969	1.0131	1.1460	.0942
40 Days					
Cortex	.6458	.8215	1.0212	1.0731	.0920
Stele	.1476	.1948	.2404	.2215	.0248
Total	.7934	1.0163	1.2616	1.2946	.1107

sections. The radicle of L-317 is elliptical in transverse section (Fig. 14), that of Bl-349 is somewhat elongated (Fig. 15), and the reciprocal hybrids are intermediate (Figs. 16, 17). Radicles of the inbreds and hybrids differ chiefly in relative cell enlargement. The chief region of cell enlargement is the proximal portion of the radicle. Therefore, the region chosen for the comparison of measurements was the most proximal transverse section in which the radicle is separated from the coleorhiza by a cleft. Comparisons between transverse sectional areas of the radicles appear in Table 11.

Thirty days after pollination the inbreds L-317 and Bl-349 do not differ significantly in transverse sectional area of the cortex of the radicle. The inbred L-317 is significantly larger than Bl-349 in transverse sectional area of the stele. Thirty-five days after pollination the inbreds are not significantly different with respect to the areas of either stele or cortex. Forty days after pollination the inbred Bl-349 is significantly larger than L-317 in area of cortex, but not significantly different in area of stele (Figs. 18, 19).

Thirty days after pollination the hybrids are not significantly different in transverse sectional area of either stele or cortex. Thirty-five days after pollination, Bl-349 x L-317 is significantly larger in transverse sectional area of the cortex, but not significantly different in stelar area.

At 40 days, Bl-349 x L-317 is significantly larger than its reciprocal in both stele and cortex (Figs. 20, 21).

Mean transverse sectional areas of the radicles of the inbreds and reciprocal hybrids, and the least significant differences appear in Table 12.

Thirty days after pollination, both hybrids are significantly larger than either parent in transverse sectional area of both cortex and stele. Thirty-five days after pollination there are no significant differences in the

TABLE 11

ANALYSIS OF VARIANCE OF TRANSVERSE SECTIONAL AREAS OF THE RADICLES, 30, 35, AND 40 DAYS AFTER POLLINATION

	M.S. Between Groups (3 d.f.)	M.S. Within Groups (36 d.f.)	M.S. for Testing the Difference Between	
			Inbreds (1 d.f.)	Hybrids (1 d.f.)
30 Days				
Cortex	14300*	2500	224	1008
Stele	3715	612	3976†	461
Total	30245	4937	6090	2832
35 Days				
Cortex	16010	2459	9418	18362‡
Stele	1800	474	572	1140
Total	25072	4473	5346	28652‡
40 Days				
Cortex	61516	1866	98420†	52429‡
Stele	3469	291	29	3976‡
Total	83279	3015	95082†	85282‡

* All entries $x10^{-6}$
† F larger than tabulated 5% value
‡ F larger than tabulated 1% value

cortical areas of the hybrids and their parents, but Bl-349 x L-317 still exceeds its female parent in stelar area. Forty days after pollination, L-317 x Bl-349 is significantly larger than L-317 in cortical area, whereas Bl-349 x L-317 is significantly larger than Bl-349 in stelar area.

The radicles of the inbreds and hybrids were also compared on the basis of the total transverse sectional area of the metaxylem elements. These comparisons appear in Table 13.

At 30 and 35 days the inbreds do not differ significantly in the total transverse sectional area of metaxylem elements, neither do the hybrids

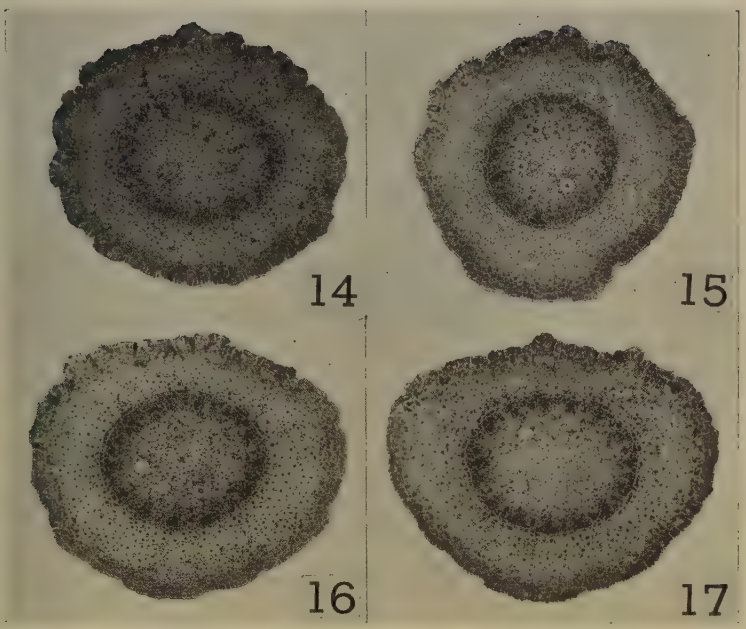

FIG.

14. Transverse section of the radicle of inbred L-317, showing relatively large stele, 30 days. x34
15. Transverse section of the radicle of the inbred Bl-349, showing relatively small stele, 30 days. x34
16. Transverse section of the radicle of the hybrid L-317 x Bl-349, 30 days. x34
17. Transverse section of the radicle of the hybrid Bl-349 x L-317, 30 days. x34

differ significantly. However, at 40 days, the hybrid Bl-349 x L-317 is significantly larger than its reciprocal.

Mean transverse sectional area of metaxylem elements of the inbreds and their reciprocal hybrids, and the least significant differences are given in Table 14.

The hybrid Bl-349 x L-317 significantly exceeds its female parent at all ages, whereas the reciprocal, L-317 x Bl-349, exceeds its female parent significantly to 30 days.

DEVELOPMENT OF SEMINAL ROOTS

The region of seminal root development lies just above the scutellar node. These roots do not emerge perpendicular to the main axis of the embryo; therefore, transverse sections show them in oblique view, and

TABLE 12

MEAN TRANSVERSE SECTIONAL AREA OF RADICLES, 30, 35, AND 40 DAYS AFTER POLLINATION (in sq. mm.)

	L-317	L-317 x Bl-349	Bl-349 x L-317	Bl-349	L. S. D.
30 Days					
Cortex	.4229	.4770	.4912	.4162	.0061
Stele	.1692	.1762	.1858	.1410	.0300
Total	.5921	.6532	.6770	.5572	.0854
35 Days					
Cortex	.5289	.5653	.6259	.5723	.0603
Stele	.1953	.2016	.2167	.1846	.0265
Total	.7242	.7669	.8426	.7569	.0814
40 Days					
Cortex	.5362	.6132	.7156	.6765	.0524
Stele	.2078	.2178	.2460	.2054	.0207
Total	.7440	.8310	.9616	.8819	.0668

it is not possible to make comparable sectional area measurements. Visual observations were made as to the time of seminal root initiation, enlargement, and differentiation.

Thirty days after pollination, L-317 does not show any indications of seminal root initiation. The inbred Bl-349, at 30 days, has two seminal root primordia on the posterior side of the embryonic axis. Seminal roots are evident in Bl-349 x L-317 at this date but they are lacking in the reciprocal.

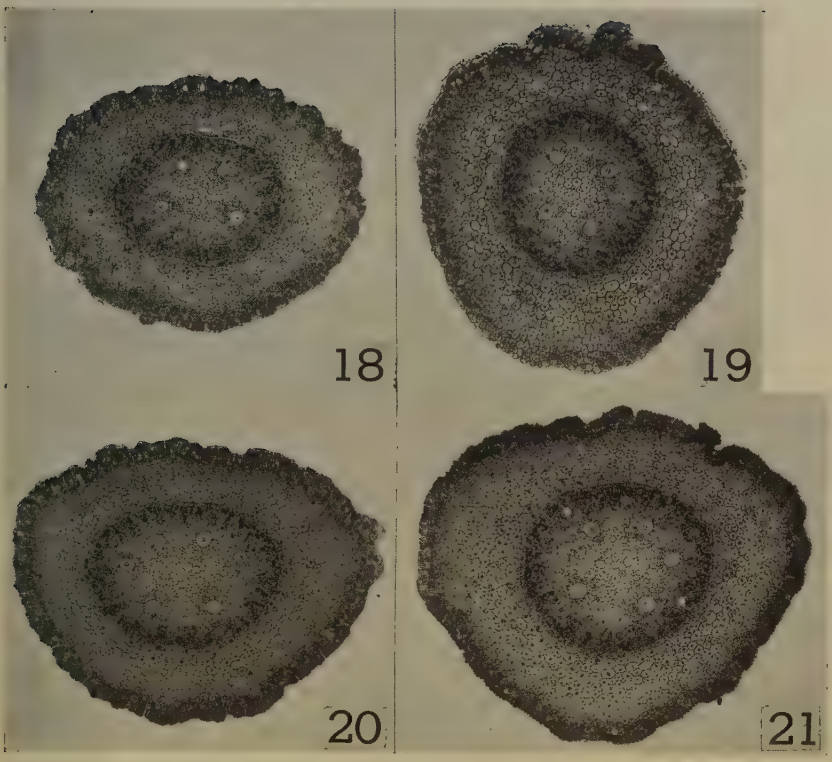

Fig.
18. Transverse section of the radicle of inbred L-317 showing relatively small cortex, 40 days. x34
19. Transverse section of the radicle of the inbred Bl-349, showing relatively large cortex, 40 days. x34
20. Transverse section of the radicle of the hybrid L-317 x Bl-349, 40 days. x34
21. Transverse section of the radicle of the hybrid Bl-349 x L-317, 40 days. x34

At 35 days, seminal roots are still absent in L-317 but have appeared in the hybrid L-317 x Bl-349. The inbred Bl-349 and the hybrid Bl-349 x L-317 have two well-formed seminal roots, and both the inbred and the hybrid have a less advanced seminal root on the anterior side of the axis.

Forty days after pollination the inbred L-317 shows evidence of seminal root initiation in the pericycle. This activity is recognized as an area of densely stained cells (Fig. 22). No recognizable histogens are evident. The inbred Bl-349 has three well-developed seminal roots

TABLE 13

Analysis of Variance of Total Transverse Sectional Areas of Metaxylem Elements, 30, 35, and 40 Days After Pollination

	M.S. Between Groups (3 d.f.)	M.S. Within Groups (36 d.f.)	M.S. for Testing the Difference Between	
			Inbreds (1 d.f.)	Hybrids (1 d.f.)
30 Days	7150650	1441544	37411	605
35 Days	7250298	1546740	14742	218196
40 Days	12161636	1303767	1687805	19902120*

* F larger than tabulated 1% value

(Fig. 23). The seminal root on the anterior face does not appear in this figure because it is at a different level. The calyptra, periblem, and plerome are distinct in the two posterior seminal roots, whereas the anterior seminal root is less advanced. The seminal roots of the hybrids do not appear to be as large as those of Bl-349, but they are approximately equal to Bl-349 in histological development. The hybrid Bl-349 x L-317 (Fig. 25) is more advanced than its reciprocal (Fig. 24).

The inbreds L-317 and Bl-349 are strikingly different in the time of initiation of seminal roots, and both hybrids approach the more vigorous parent.

TABLE 14

Mean Total Transverse Sectional Area of Metaxylem Elements, 30, 35, and 40 Days After Pollination (in sq. microns)

	L-317	L-317 x Bl-349	Bl-349 x L-317	Bl-349	L.S.D.
30 Days	6524	8025	8036	6610	1460
35 Days	7739	9074	9283	7685	1513
40 Days	8471	8983	10980	9052	1389

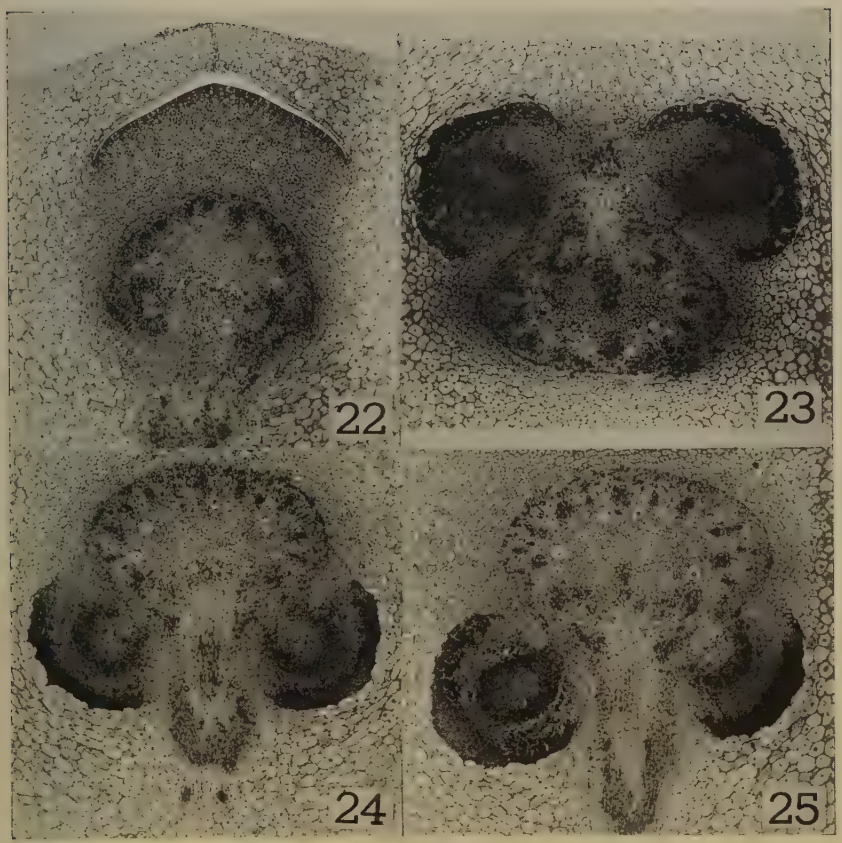

FIG.

22. Transverse section of the basal portion of the first internode of inbred L-317, showing region of seminal root initiation, 40 days. x34
23. Transverse section of the basal portion of the first internode of inbred Bl-349, showing two well-developed seminal roots, 40 days. x34
24. Transverse section of the basal portion of the first internode of hybrid L-317 x Bl-349, 40 days. x34
25. Transverse section of the basal portion of the first internode of hybrid Bl-349 x L-317, 40 days. x34

DISCUSSION

Previous investigations of heterosis in maize have shown that the dry weight of hybrid embryos may be less than, equal to, or greater than the embryo weight of parent inbreds. The present study was undertaken to explore the possibility that heterosis may have histological or morphological expression in the maize embryo.

The two inbreds L-317 and Bl-349 were found to differ markedly in embryo development as early as five days after pollination, and by the fifteenth day the embryo of Bl-349 is larger and more advanced than that of L-317. The two hybrids are intermediate and tend to resemble their female parent.

The two inbreds chosen for study differ markedly in plumule development with respect to time of leaf initiation, activity of marginal leaf meristems, and size of plumular organs as indicated by transverse sectional area.

The hybrid embryos, L-317 x Bl-349, grown on the same ears with the less vigorous parent L-317, surpass the inbred embryos of the maternal parent in plumular development throughout the entire period of embryo growth. The expression of heterosis in this instance consists of increased leaf initiating activity of apical meristems, increased activity of marginal leaf meristems, and increase in total plumule size, as indicated by transverse sectional area. This hybrid, L-317 x Bl-349, does not equal its male parent during early growth, but is approximately equal to the male parent at 40 days.

The hybrid embryos of the reciprocal cross, Bl-349 x L-317, grown on the same ears with the more vigorous inbred Bl-349, were found to be inferior to the embryos of the maternal parent in plumular development until approximately 35 days after pollination. This hybrid surpasses its male parent throughout the entire period of plumular development on the basis of all the criteria used in judging comparative development.

The differences between the inbreds in the observed rate of leaf initiation are in agreement with the comparative development of these lines after germination. The inbred L-317 is a late maturing inbred. The hybrid L-317 x Bl-349 and its reciprocal differ in rate of leaf initiation. Since these two hybrids are genetically identical the difference may be the result of maternal influence (15) (8). The fact that L-317 x Bl-349 equals the more vigorous parent in leaf initiation may indicate that those genes from Bl-349 which influence this growth process are dominant.

The fact that Bl-349 x L-317 exceeds its more vigorous parent in total transverse sectional area of plumular organs may be the result of the additive or complimentary effects of genes from both parents (15). An alternative explanation may be that this difference is due to the effect of hybrid endosperm.

The inbreds used in this study are strikingly different in transverse sectional area of the cortex and stele of the first internode. The

hybrid embryos, Bl-349 x L-317, grown on the same ears with the more vigorous inbred Bl-349, are equal to the embryos of the maternal inbred in stelar enlargement, but do not become equal in cortical enlargement until after 35 days. The hybrid embryos, L-317 x Bl-349, grown on the same ears with the less vigorous inbred L-317, surpass the inbred embryos in transverse sectional area of the cortex and stele of the first internode.

Maternal influence in the development of the first internode is evidenced by the fact that the hybrid embryos, grown on the more vigorous inbred, exceed the embryos of the reciprocal hybrid in the transverse sectional area of the stele and cortex at all ages.

The inbreds L-317 and Bl-349 are not obviously different in the histological development of the radicle. Differences which do exist consist largely in the relative enlargement of the stele and cortex. With respect to stelar enlargement the inbred L-317 is superior to Bl-349 at 30 days, but does not maintain this advantage. The inbred attains a slight superiority in transverse sectional area of the cortex by 40 days after pollination.

The embryos of the hybrid L-317 x Bl-349 exceed those of the inbred L-317 in transverse sectional area of the cortex by 30 days after pollination. However, this hybrid, L-317 x Bl-349, does not exceed its maternal parent at any age. The embryos of the hybrid Bl-349 x L-317 exceed those of the inbred Bl-349 in transverse sectional area of both stele and cortex by 30 days. By 35 days after pollination, the hybrid Bl-349 x L-317 has lost its superiority in transverse sectional area of the cortex, but exceeds its maternal parent in transverse sectional area of the stele at all ages.

The above comparisons indicate that L-317 contributes favorable genes for the growth of the stele, whereas Bl-349 contributes favorable genes for the growth of the cortex. Therefore, heterosis in the radicle is the result of the combined effects of favorable genes that are contributed by both parents.

In seminal root development, the outstanding difference between the inbreds is in the time of root initiation. Seminal root development in Bl-349 is more advanced than in L-317, whereas both hybrids approach the more advanced parent. The genes which influence early initiation of seminal roots appear to be dominant. A maternal effect is indicated by the fact that seminal root development in hybrid embryos that are grown on the same ear with the more advanced inbred embryos, exceeds the seminal root development in hybrid embryos that are grown on the less advanced inbred.

SUMMARY

The present investigation was undertaken to explore the possibility that heterosis may be expressed in the morphological or histological development of the maize embryo.

The two dent inbred lines L-317 and Bl-349, and their reciprocal

hybrids, were chosen for detailed study because preliminary observations revealed that these inbreds differ strikingly in embryological development.

A split-ear pollination technique was employed, whereby inbred and hybrid embryos were grown on the same ear. Embryos of each inbred and of each of the reciprocal hybrids were studied 5 days after pollination, and at 5-day intervals thereafter until 40 days.

Comparisons were made on the basis of visual study of longitudinal and transverse sections of the embryos at 5, 10, and 15 days, and serial transverse sections of embryos from 20 to 40 days after pollination. Four levels were chosen as the chief regions to be compared. These regions were: the transverse section of the plumule at the level of the apical meristem, a transverse section near the basal portion of the first internode, the transverse section at the level of seminal root initiation, and the transverse section of the radicle at the most proximal region at which it is separated from the coleorhiza by a cleft. Visual observations were supported by measurements of transverse sectional area and by statistical analysis.

Histological expressions of heterosis are evident in plumular development. Both reciprocal hybrids surpass the less vigorous inbred in the rate of leaf initiation, in the activity of marginal leaf meristems, and in the transverse sectional area of plumular organs. Hybrid embryos grown on the same ear with the more vigorous inbred surpass the more vigorous inbred 40 days after pollination, but the reciprocal hybrid is still intermediate in development at this age. The differences between reciprocal hybrids are interpreted as the expression of maternal effect.

Expressions of heterosis are also evident in the development of the first internode. Both hybrids exceed the less vigorous parent in the total transverse sectional area of the first internode, but neither hybrid exceeds the more vigorous parent in this respect.

The expression of heterosis in the radicle is quite evident. The inbreds do not differ significantly in total transverse sectional area of the radicle at 30 days, but L-317 is larger than Bl-349 in the area of the stele. By 40 days, Bl-349 exceeds L-317 in the transverse area of the cortex. These observations, as well as the fact that both reciprocal hybrids exceed either inbred parent in transverse sectional area of both stele and cortex at 35 days, indicate that L-317 contributes favorable genes for stelar development, whereas Bl-349 contributes favorable genes for cortical development. The observed heterosis is the result of favorable genes from both parents.

The comparative development of seminal roots also provides evidence of heterosis. The inbreds are obviously different in the time of initiation of seminal roots. The reciprocal hybrids are intermediate, but they tend to approach the more advanced parent. In the size of seminal roots and in the differentiation of histogens, both hybrids far exceed the less advanced inbred. Neither hybrid appreciably surpasses the more advanced inbred in seminal root development.

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THE DEVELOPMENT AND STRUCTURE OF THE VEGETATIVE AND REPRODUCTIVE ORGANS OF KUDZU, PUERARIA THUNBERGIANA (SIEB. AND ZUCC.) BENTH.[1]

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The kudzu bean, *Pueraria thunbergiana* (Sieb. and Zucc.) Benth., is a vigorous, woody perennial vine, native to Japan and China. The plant was introduced into the United States from the Orient in 1876, when it was exhibited at the Philadelphia Centennial. It is a valuable forage legume, and it is also used for soil improvement, for erosion control, and as an ornamental.

Recent studies by McKee and Stephens (17) have shown that kudzu thrives best in the humid southeastern states. It is grown to some extent in the East, Southwest, Midwest, and Far West, but it is best adapted to states south of Virginia and Kentucky.

The present study of the development and structure of the vegetative and reproductive organs of kudzu was undertaken to furnish a basis for studies in the breeding, cultivation, and utilization of kudzu.

REVIEW OF PERTINENT LITERATURE

Solereder (24) described some features of the anatomy of kudzu, particularly the origin of cork in the second to sixth subepidermal cell layers. Burkart (5) reported that the tuberous roots, from which starch is obtained, develop from adventitious roots. Tabor (28) found that seedlings have several advantages over "crowns" for propagation. The seedlings can be produced in one growing season, the yield per acre is higher, and lifting, storage, transportation, and planting are less difficult than with "crowns." Tabor's (27) studies of production in Virginia, North Carolina, South Carolina, Georgia, Florida, Alabama, and Mississippi showed that kudzu vines growing over bushes, trees, fences, or other supports produce seed most abundantly. The best-seeding plants have ten to twelve seeds per pod and twenty to thirty pods per cluster.

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Within the past few years, agronomic studies have been made in the United States, South Africa, Cuba, Australia, and other countries to determine the economic value of the kudzu bean. Degener (8) found that "ko-pu" fiber of good quality can be prepared from the bark of the young shoots of kudzu. Pierre and Bertram (20) found that increasing the number of cuttings of kudzu reduces its production of food reserves. Among the more recent studies are those by Tabor (26) and Arnold (1) on the cultivation of kudzu; Bailey and Mayton (2), Brink (3), Cates (7) and Swabey (25) on its value for erosion control; McKee and Stephens (17), Calvino (6), McMartin (18), Pieters (21, 22) and Tracy (29), on its value as a forage crop.

MATERIALS AND METHODS

The kudzu plants used in this study were obtained from eroded land along a stream which flows through the truck garden of Tuskegee Institute, Tuskegee, Alabama. The plants were between eight and ten years old and had been grown from transplants.

Seeds were obtained through the courtesy of Dr. Roland McKee and Dr. M. F. Spaulding, who made the collections in the vicinities of Beltsville, Maryland, and Tuskegee, Alabama, respectively. The seeds were prepared for germination by soaking in concentrated sulphuric acid for twenty to thirty minutes and then thoroughly washing in tap water.

For anatomical sections and for some cytological studies, tissues were killed in modifications of the Nawaschin (Craf) formula and embedded in paraffin. Anatomical preparations were stained in safranin-fast green, and cytological sections were stained in gentian violet-iodine or iron-alum hematoxylin.

OBSERVATIONS

GROSS MORPHOLOGY OF THE PLANT

Kudzu is a coarse, hairy, stoloniferous vine. The stems, which may attain a length of more than seventy feet during a growing season, are prostrate or climb over brush, shrubs, trees, fences, or other supports. The pubescent, cylindrical stems are usually less than a centimeter in diameter throughout their length (Fig. 1). When the stems are in direct contact with moist soil, rooting occurs at the nodes (Fig. 2). The seedling stem grows vertically a few inches above the ground and then bends at an angle of almost ninety degrees to a prostrate position.

The leaves are alternate, trifoliate, with the lateral leaflets 8 to 10 cm. in width and 12 to 15 cm. in length. The median leaflet is usually 5 to 7 cm. wide and 12 to 20 cm. long (Fig. 3). The length of the petioles ranges from 2 cm. near the stem tip to 25 cm. on the mature portions of the stem. The petiole is enlarged at the base for a distance of 5 mm. or more, forming a definite pulvinus. Just above the pulvinus, the petiole is rounded in cross section, except for a groove on the adaxial surface. The leaflets have short, thick stalks and their margins are

lobed or entire. The netted venation has three principal veins in each leaflet. The stipules are membranous, ovate, and acute.

Observations were made on the movements of the leaves of kudzu plants growing in pots and in the field. Bending occurs at the pulvinus at the base of the petiole and at the base of each leaflet. The petioles rise to the erect position during the period from 7 A.M. to 3 P.M and begin to change to the nocturnal position from 3 P.M. to 7 P.M. About 1 P.M. the lateral leaflets fold so that they almost touch. The terminal leaflet bends until its surface touches the vertical edges of the lateral leaflets.

The root system begins as a tap-root, developed from the primary root of the seedling. Abundant, fibrous lateral roots develop early on the primary root. Adventitious roots arise from stolons in contact with moist soil.

Kudzu begins to bloom during the middle of July in the region of Tuskegee, Alabama. The pedicellate flowers are borne in 2–3 flowered dicasial clusters along a raceme-like axis. The inflorescence as a whole appears to be an axillary raceme located near the end of the stem (Fig. 5). The number of flowers in an inflorescence varies from a few to over three hundred. The peduncles and pedicels are pubescent. The pedicels are approximately as long as the calyx. The calyx is tubular and is subtended by two lanceolate bractlets about 5 mm. long. The lanceolate lobes of the calyx are longer than the tube. The basal lobes of the calyx are somewhat longer and narrower than the others (Fig. 6). The dark red-purple calyx tube is densely covered with tan-colored hairs.

The dimensions of the five petals are approximately as follows: the upright upper petal (standard) is 15 mm. long and 11 mm. wide, and has a claw 3 mm. long and 1 mm. wide; the lateral petals (wings) are 10 mm. long and 0.5 mm. wide, with a claw 5 mm. long and 0.5 mm. wide; the keel petals are 12 mm. long and 6 mm. wide, with a claw 5 mm. long and 0.5 mm. wide.

The ten stamens are of unequal length. The filaments of nine stamens are fused and form a tube around the pistil, whereas the tenth stamen is free. The filaments are attached to the backs of the anthers. The anthers are about 1 mm. long and 0.4 mm. wide. The pollen grains are almost spherical and are relatively rough.

The unicarpellate pistil is about 19 mm. long. The ovary is about 5 mm. long and 1 mm. thick, and has long, white hairs. The style is 14 mm. in length, curved and tapered at the apex. Nectaries are located at the base of the staminal tube on either side of the free stamen.

The formation of pollen takes place very early and the anthers dehisce while the corolla is still closed. Anthesis progresses from the base of the inflorescence toward the apex and requires twenty-two to thirty days for completion. The flowers of the kudzu are adapted for cross-pollination by honey bees and bumble bees.

Each inflorescence bears from ten to thirty pods. They are yellow-

FIG. 1. Terminal portion of stoloniferous stem.
FIG. 2. Adventitious roots at node in contact with moist soil.
FIG. 3. Alternate trifoliate leaves.
FIG. 4. Terminal portion of runner showing inflorescenses in axils of trifoliate leaves.
FIG. 5. Arrangement of flowers in an inflorescence.
FIG. 6. Organs of dissected flower.

green, with numerous long, reddish-brown hairs (Fig. 5). The pods are 5 to 8 cm. long and about 1 cm. wide. Each pod has from three to ten seeds.

The seeds are kidney-shaped and average about 3 mm. in length and 2 mm. in width. The surface of the testa is lustrous and usually reddish-brown or gray with black specks. In the center of the concave surface there is a light-colored hilum 0.5 mm. in length, and bordered by a raised margin. The seeds have a hard and impermeable seed coat which becomes permeable when soaked in concentrated sulphuric acid for twenty to thirty minutes. After this treatment seeds germinate in less than twelve hours.

The relatively large seedling has two green, epigeal cotyledons. The hypocotyl is long and merges gradually into the primary root. The seed coat is not carried on the tip of the cotyledons but remains in the soil. The fleshy cotyledons are approximately 8.5 mm. long and 4.5 mm. wide. Their petioles are broad and united at their bases to form the cotyledonary sheath. The hypocotyl varies greatly in length, and is about 0.3 mm. in diameter. It is slightly swollen near the collet, the region of transition between the root and the hypocotyl.

The primary root is much smaller in diameter than the hypocotyl. Lateral rootlets develop very early during the growth of the seedling, and by the time the plumule appears between the cotyledons, at least two rows of rootlets can be distinguished. By the time the second compound leaf appears, three rows of rootlets have developed.

The cotyledons enlarge rapidly during the first few days. The first leaves are opposite and simple, and generally appear about eight days after the cotyledons have become divergent. The laminae of the first leaves are pubescent, entire, acuminate, slightly cordate, about 7 mm. in length and 6 mm. in width. The leaves that emerge subsequently are alternate, trifoliate, and pubescent.

ANATOMY OF THE VEGETATIVE ORGANS

The Stem. The stem apex has a tunica of two layers of cells, in which the cells divide predominantly in an anticlinal plane, and a central corpus in which the cells divide in random planes.

The primary vascular system consists of a ring of closely spaced collateral bundles, separated by narrow, parenchymatous rays. The primary phloem contains sieve tubes, companion cells, and scattered parenchyma. The primary xylem consists of large vessels, small tracheids, and some parenchyma. Pericycle and endodermis are not well defined in the very young stem.

Interfascicular cambium arises relatively early and cambial activity soon produces an essentially continuous cylinder of secondary xylem, consisting of vessels, tracheids, and parenchyma. Secondary phloem occurs in discontinuous strands of vascular and ray elements. Some secondary phloem sclerenchyma is present, in addition to the vascular and parenchymatous elements (Fig. 7).

The pericycle differentiates into a broad, nearly continuous band of sclerenchyma, interrupted by narrow zones of parenchyma. The endodermis is a single layer of cells, which contain starch in the young stem, but in the older stem the starch disappears and considerable amorphorus, stainable material accumulates.

The cortex consists of a broad inner zone of parenchyma, a band of chlorenchyma, and discontinuous strips of collenchyma. The lightly cutinized epidermis bears abundant trichomes. Periderm arises in the second or third subepidermal layer of the cortex. The cork cambium produces typical cork and a limited amount of parenchymatous phelloderm.

The Root. A longitudinal section of the apex of the emerging radicle

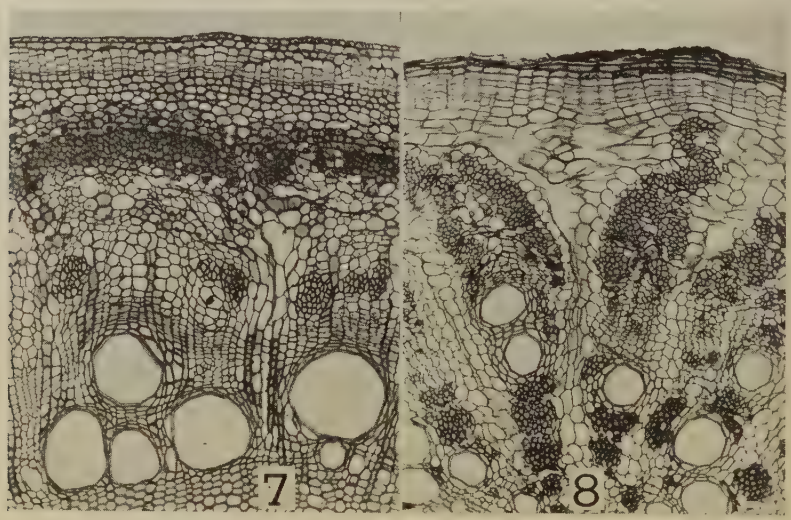

FIG. 7. Sector of transverse section of stem with prominent secondary vessels, well differentiated pericyclic sclerenchyma, and some periderm activity. X62.
FIG. 8. Sector of transverse section of root that has had considerable secondary activity and periderm formation. X62.

shows a root cap, and three distinct histogens, the dermatogen, periblem, and plerome. The promeristem is of the "open type," in which a broad, transverse initiating zone produces the root cap and the primary tissue systems of the root. Periclinal divisions occur in the cortical derivatives of the initiating zone and determine the thickness of the cortex. After cell division ceases in the inner region of the periblem, the endodermis becomes differentiated from the innermost layer of cells.

The primary root has an exarch, tetrarch stele. The protophloem and protoxylem elements differentiate essentially simultaneously. The

preicycle and the endodermis become well differentiated early. The large protoxylem cells differentiate rapidly and develop a thick, lignified secondary wall. There is considerable parenchyma between the xylem and the phloem strands. The sieve tubes of the metaphloem are much larger in diameter than those in the protophloem. The diameter of the metaxylem cells is also greater than that of the protoxylem cells (Fig. 8).

After the primary tissues of a root are clearly differentiated, lateral roots arise endogenously from the meristematic cells of the pericycle, opposite the protoxylem points. Cambium originates between the primary xylem and phloem at approximately the same level of root development as lateral root initiation. Cambial activity produces a core of secondary xylem and a comparatively thin layer of secondary phloem. The primary cortex is cut off early from the vascular system by the formation of a periderm of pericyclic derivation, and the cortex soon sloughs off.

The Leaf. The upper and lower epidermal layers of the kudzu leaf are composed of closely fitted thick-walled rectangular cells, 4 to 15 microns in length and 4 to 7 microns in width. In the region of the midrib the cell walls are considerably reinforced. Cuticle is present on both surfaces of the leaf. Stomates are abundant on both surfaces, but they are more numerous on the lower surface. The palisade tissue makes up about half of the thickness of the mesophyll. The very loose spongy parenchyma consists of two layers of cells. The collateral vascular bundle in the midrib has cambium and some secondary tissue, whereas the small veinlets exhibit no secondary growth. Mechanical tissue is present in strands on the abaxial side of the midrib.

THE FLOWER

A flower arises as a dome-shaped primordium in the axil of a bract. The first perianth whorl to appear is the calyx, which consists at first of five separate lobes but which later becomes gamosepalous. Very early in floral ontogeny, the calyx arches over the primordia of the other floral structures. The petal primordia arise alternate with the lobes of the calyx. The keel petals develop first, wing petals next, and the standard last. The stamens appear soon after the initiation of petal primordia. Five stamens develop in advance of the other five. Pollen quartets are present in the first set when meiosis occurs in the later set. The filaments of all ten stamens are free a few millimeters below the anthers, but below this level nine filaments are fused into the staminal tube, whereas the tenth stamen remains free along its entire length.

The development of the anthers in kudzu follows the pattern that has been described in other members of the Leguminosae. Hypodermal cells of the young anther undergo periclinal divisions and produce a parietal layer and the archesporial initials. The parietal cells undergo a second division and give rise to an outer layer of daughter cells,

which become a part of the wall tissue, and an inner layer, the tapetum. At this stage of development the cells of the tapetum are much larger than the archesporial cells. The anther wall finally consists of an epidermis and a zone of two or three layers of parenchymatous cells. The archesporial cells multiply and produce the four columns of polyhedral microsporocytes. After the two divisions of meiosis, the microspores become delimited by furrowing of the cytoplasm of the sporocyte. The spherical mature pollen grains have roughened walls.

The carpel arises at the apex of the floral axis as a dome-shaped primordium. In the early stages of development the carpel is open along the ventral surface, as seen in cross section. The carpel margins become completely united before ovule formation.

The ovules arise as dome-shaped primordia along the margins of the ventral suture. The 9 to 13 ovules are arranged alternately in two rows. Many of the ovules abort. Growth of the ovules is most rapid on the basal side, consequently they curve toward the stylar end of the ovary and become campylotropous. The integuments appear simultaneously (Fig. 9). The fully developed outer integument consists of three cell layers, except in the region of the micropyle where it becomes considerably thicker by periclinal divisions.

As many as four sporogenous cells were observed in some ovules, but usually only one is functional. A linear quartet of megaspores is produced, and the surviving chalazal megaspore gives rise to the eight-nucleate female gametophyte, which consists of three ephemeral antipodals, two polar nuclei, the egg, and two synergids (Fig. 10).

THE SEED

Three to four days after fertilization the proembryo consists of a short, massive suspensor of several tiers of cells, and a terminal globular portion (Fig. 11). At this stage, the endosperm immediately surrounding the embryo is cellular, whereas the endosperm in the chalazal region is still in the free nuclear condition. The nucellus has become almost completely absorbed at the micropylar end and along the sides. The nucellus at the chalazal end undergoes absorption and the inner integument becomes reduced to one layer of cells along the sides of the ovule.

The organs of the embryo are discernible as early as ten days after pollination. The buds in the axils of the cotyledons, the hypocotyl, and the radicle are well defined, and procambial strands are clearly recognizable. The endosperm is limited to a thin layer of cells along the sides of the embryo sac. The endosperm of the mature seed constitutes an unbroken covering of two to several cell layers around the embryo. The mucilaginous endosperm absorbs water readily and becomes swollen.

The seed coat is almost entirely the product of the outer integument. The transformation of the outer integument into the seed coat begins in the outer epidermal cells in the micropylar and chalazal regions. Immediaely following fertilzation, the cells of the outer integ-

ument begin rapid radial elongation. They continue to elongate until their length in the region of the micropyle, at the time of maturity, is approximately nine times their length at the time of fertilization. A similar elongation of the subepidermal cells takes place later along the sides of the developing seed. These subepidermal cells constitute the malpighian layer. The domes and the light-line present in other legume seeds are not evident in kudzu. The length of a fully mature malpighian cell is approximately four and one-half times its width (Fig. 12).

Soon after the malpighian layer begins to differentiate, the next inner subepidermal layer differentiates into the osteosclereid layer. This layer consists of one row of I-shaped cells which are uniform in size and length. The osteosclereid layer envelops the seed except in the region of the hilum, where the cells undergo very little modification.

The nutritive zone consists of several layers of cells beneath the osteosclereids. These cells are irregular in shape, their walls are slightly thickened, and they contain chloroplasts. The nutritive layer has its origin in the nucellus.

The hilum is encircled by an arillate rim, which may function to absorb water. The strophiole is a small, elongated depression near the micropyle.

DISCUSSION

The hypocotyl of kudzu varies in length as in white clover, in which Erith (9) reported that the length of the hypocotyl is influenced by the depth of planting, intensity of light, and other external conditions.

The flowers of the kudzu develop essentially as in other members of the Leguminosae. The sequence of initiation is sepals, petals, stamens, and pistil, as reported by Guard (12) for *Soja max* by Goebel (10) for *Phaseolus,* and by Grégoire (11) for *Lathyrus, Trifolium,* and *Lupinus.*

Coalescence of the carpel margins occurs as described by Goebel (10) in *Phaseolus* and in other legumes by Bugnon (4) in *Lathyrus, Trifolium* and *Lupinus,* and by Guard (12) in *Soja max.* Grégoire (11), however, did not find any distinct line between the carpellary margins in *Lathyrus.*

The presence of four rows of cells of nucellular tissue in kudzu is similar to the condition found by Martin (15) in *Trifolium* and *Medicago sativa.* The destruction of the nucellus by the developing embryo sac in the kudzu differs from the condition found by Reeves (23) in *Medicago sativa,* by Young (31) in *Melilotus alba,* and by others. These investigators reported a complete destruction of the nucellus over the micropylar end of the embryo sac by the time of fertilization.

The occasional occurrence of more than one megasporocyte was reported by Reeves (23) in *Medicago sativa.* There is evidence that more than one megasporocyte may function in kudzu. In a number of ovules of kudzu, two embryo sacs, in the early stages of development, were observed. Martin (15) found that two rows of megaspores may occur

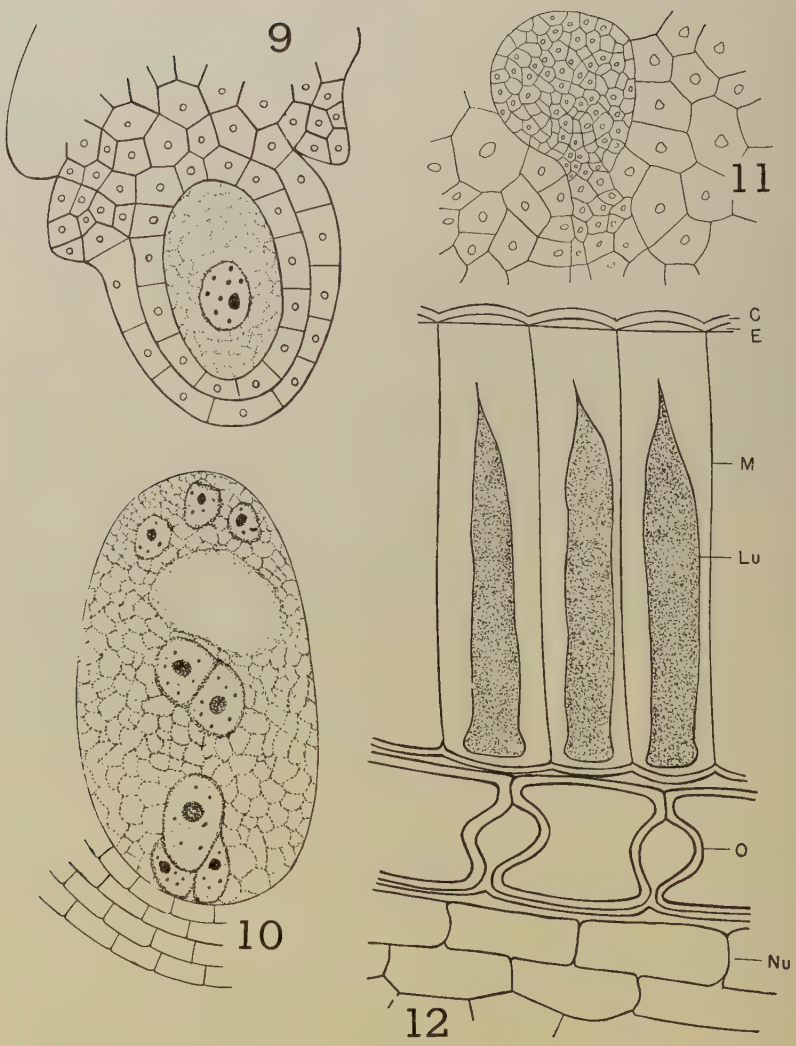

FIG.

9. Longitudinal section of ovule showing megasporocyte and the initiation of integuments. X310.
10. Female gametophyte showing antipodals, polars, synergids, egg, and four rows of nucellar tissue. X310.
11. Proembryo and a portion of the endosperm. X276.
12. Detail of seed coat, showing: C, cuticle; E, epidermis; M, malpighian cell; Lu, lumen; O, osteosclereid layer; Nu, nutritive layer. X276.

in the same nucellus in *Medicago sativa,* but usually not more than one mature embryo sac was found. However, Martin and Watt (16) reported the occurrence of the two normal embryos in a seed of *Medicago sativa* and in a seed of *Melilotus alba.* Jönsson (14) also found more than one functional embryo sac in *Trifolium pratense.*

The early disappearance of antipodals in kudzu resembles the condition reported by Martin (15) in *Trifolium, Medicago sativa,* and *Vicia americana,* and Reeves (23) noted a similar behavior in *Medicago sativa.*

In the region of the hilum of kudzu there are two palisade epidermal layers. They are similar to those described by Winton (30).

The cells of the nutritive layer in the seeds of kudzu contain chloroplasts, which supports the statement of Pammel (19), that in *Strophostyles pauciflorous* the starch in the nutritive layer serves to nourish the growing seed.

In some hard seeds, water enters the interior of the seed through the strophiole in the initial stage of germination. This has been demonstrated by Martin and Watt (16) for *Melilotus alba.* The seed of kudzu has a schlerenchymatous mass of cells below the hilum, resembling the structure found in *Pisum sativum,* illustrated in Hayward's Figure 174 (13).

SUMMARY

The present study of the development and structure of the vegetative and reproductive organs of kudzu was undertaken to furnish a basis for studies in breeding, cultivation, and utilization of the plant.

Kudzu is a coarse, hairy, stoloniferous vine with alternate, trifoliate compound leaves, and racemose, papilionaceous flowers. Twenty or thirty days are required for the completion of anthesis in one raceme.

The order of initiation of primordia of floral organs is, sepals, petals, stamens, and carpel.

The ovules arise as dome-shaped primordia along the inner margins of the ventral suture. The nine to thirteen campylotropous ovules develop simultaneously, arranged alternately in two rows.

The sporogenous cell functions as the megasporocyte, which undergoes meiotic divisions and produces a linear quartet of megaspores. Three of the megaspores disintegrate, and the surviving chalazal megaspore gives rise to the female gametophyte.

The female gametophyte consists of an egg, two synergids, two partially coalesced polar nuclei, and three more or less disintegrated antipodal nuclei. The nucellus soon becomes absorbed at the micropylar end and along the sides.

The proembryo has a short, massive suspensor of several tiers of cells. The mature embryo consists of two cotyledons, a simple leaf, a compound leaf, the hypocotyl, and a large radicle. The endosperm of the mature seed constitutes an unbroken covering of two to several cell layers around the embryo.

The seed coat is almost entirely the product of the outer integument. The malpighian layer has no domes or light-line. The osteosclereid layer

consists of one row of I-shaped cells. This layer is continuous except in the region of the hilum. The nutritive layer contains chloroplasts.

An arillate rim surrounds the hilum. The strophiole is a small, elongated depression in the region of the hilum, opposite the micropyle.

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DETERMINATION OF AMIDE NITROGEN IN VITAMINS B_{12} AND B_{12a}

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It has been reported that vitamin B_{12} contains fourteen nitrogen atoms (2). Six of these nitrogen atoms are very weakly basic in character, for they can be titrated only in glacial acetic acid solution with perchloric acid (1). The absence of primary amino nitrogen was established using the Van Slyke apparatus (3).

The difference in the infrared absorption spectra of vitamin B_{12} and the (reasonably pure) red, acid fragment produced by its hydrochloric acid hydrolysis can be explained as produced by the cleavage of acid amide groups. The present study was undertaken to determine the number of such amide groups present. A modification of the Van Slyke method was used in which the acetic acid-nitrite-sample mixture is made 2N in hydrochloric acid and the reaction allowed to run for twenty-four hours. Under these conditions the amide group yields nitrogen, the volume of which is measured (5).

EXPERIMENTAL WORK

A commercial, Van Slyke, micro amino nitrogen apparatus (A. H. Thomas Company) was employed. The reaction chamber was modified to hold twice the usual volume.

Crystalline vitamin B_{12}, obtained from E. R. Squibb and Sons of New Brunswick, N. J., was dissolved in water and the concentration determined spectrophotometrically, $E_{1\ cm.}^{1\ \%} = 204$ at 361 mμ.

Vitamin B_{12a} was prepared by hydrogenation of vitamin B_{12} and oxidation of the resulting B_{12r} (4). The crystalline B_{12a} was dissolved in water. Inasmuch as the changes which go on for some time after B_{12a} is dissolved in water render the spectrophotometric method of determining B_{12a} concentration inexact, aliquots of the solution were analyzed for cobalt. The cobalt determinations were made by the nitroso-R-salt method (6) after wet ashing.

Preliminary analyses were made on glycine, alanine, and asparagine to check the operation of the apparatus and method. Blank runs were made on the reagents, the same periods of time being used.

RESULTS

The blanks, run at intervals throughout the course of the work, gave the following: 1.017, 1.015, 1.028, 1.007, (average 1.017) mg. of nitrogen.

The analyses made on B_{12} gave the data and results shown in Table 1. The work on B_{12a} is summarized in Table 2.

An analysis was also made on chloropentamminocobalti chloride. No nitrogen above the blank was evolved.

Auxiliary experiments were performed in which samples of vita-

TABLE 1

RESULTS OF ANALYSES OF VITAMIN B_{12}

Wt. Taken mg.	Wt. N Obtained (Net) mg.	Nitrogen percentage	Number of Nitrogen Atoms
41.04	2.1136	5.15	4.96
28.04	1.4874	5.30	5.11
28.43	1.3151	4.63	4.46
62.25	3.4496	5.50	5.28
31.13	1.5353	4.93	4.75

mins B_{12} and B_{12a} were left for 24 hours in a solution of acetic acid 2N in hydrochloric acid. The solutions were then brought to *p*H 8.5 and steam distilled, the ammonia being collected and determined colorimetrically. No ammonia was obtained, showing that no appreciable acid hydrolysis occurred during the 24-hour contact with the acid.

CONCLUSIONS

Five acid amide groups are present in both vitamin B_{12} and vitamin B_{12a}.

It is quite probable that these five amide groups are the source

TABLE 2

RESULTS OF ANALYSES OF VITAMIN B_{12a}

Wt. Cobalt mg.	Wt. Nitrogen Obtained (Net) mg.	N/Co
5.013	4.140	5.14
7.330	5.725	5.39
5.987	5.087	4.95

of the five nitrogen atoms which form the ammonia liberated during the hydrochloric acid hydrolysis of vitamin B_{12}. They are probably also five of the six weakly basic groups in the molecule, the sixth titratable group being one of the benzimidazole nitrogen atoms.

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CATALYTIC BEHAVIOR OF VITAMIN B_{12a} IN THE OXIDATION OF IODIDE BY AIR

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In the course of an attempt to measure quantitatively the uptake of bromine by vitamins B_{12} and B_{12a}, it was discovered that vitamin B_{12a} catalyzes the air oxidation of iodide to free iodine.

The velocity of the reaction and the turnover number of B_{12a} in this reaction have been measured at various concentrations of B_{12a}, iodide, and sulfuric acid. Because it seemed possible that this catalytic effect is exerted through the union of B_{12r} (in which the cobalt atom is bivalent) with molecular oxygen, several experiments were conducted to determine if B_{12a} is a binuclear compound in which the cobalt atoms of two molecules of the vitamin are linked through a peroxo group.

EXPERIMENTAL WORK

1. *Reagents.* Vitamin B_{12a} was prepared by the hydrogenation of crystalline vitamin B_{12}, obtained from E. R. Squibb and Sons, New Brunswick, N. J., and air oxidation of the B_{12r} so obtained (1). The concentration of B_{12a} in this solution was determined spectrophotometrically at 352.5 mμ (phosphate buffer, *p*H 7.4, Beckman D. U. instrument). The value $E_{1\text{ cm.}}^{1\%} = 150$ was used.

Oxygen-free nitrogen was obtained by passing tank nitrogen through a train of vanadous sulfate (2).

2. *The Reaction of B_{12a} with Iodide.* B_{12a}, potassium iodide, and sulfuric acid were brought together in the complete absence of oxygen. Any iodine liberated was titrated with thiosulfate.

The reaction vessel used consisted of a wide-mouth, conical flask with two smaller necks. The large opening was closed by a four-hole rubber stopper through which were passed (a) a buret tip, (b) dropping funnel, (c) a gas inlet tube bearing a fritted glass dispersion cylinder at its lower end, and (d) a gas outlet tube leading to a water trap outside the flask. Platinum and saturated calomel electrodes were introduced through the smaller side arms.

The solution of potassium iodide was placed in the flask and, with magnetic stirring, was deaerated by the passage of a slow stream of oxygen-free nitrogen through the solution for two hours. Concurrently, dilute sulfuric acid, placed in the dropping funnel, was deaerated by bubbling through it a slow stream of oxygen-free nitrogen. The sodium thiosulfate solution, stored in a Machlett buret, had previously been

freed of dissolved oxygen in a similar manner. Immediately preceding the reaction, crystalline B_{12a} was dissolved in carefully deaerated water. Aliquots of this were introduced into the reaction vessel using a pipet previously flushed with nitrogen. Other aliquots were taken for a spectrophotometric measurement of the B_{12a} content. Sulfuric acid was then added to the reaction mixture. After an interval of several hours any iodine liberated was titrated potentiometrically with thiosulfate. The thiosulfate solution (approximately 0.001 N) was standardized potentiometrically by the titration of aliquots of a standard potassium iodate solution treated with potassium iodide and hydrochloric acid. The potassium iodate solution was prepared by weight from primary standard material.

A typical reaction mixture contained 0.0135 g. of B_{12a}, 10.00 g. of potassium iodide, and 10.0 ml. of 4.0 N sulfuric acid, all in a total volume of 235 ml.

It was necessary to take rigid precautions to exclude oxygen; in particular, the nitrogen was bubbled through the vanadous sulfate train very slowly and all rubber tubing was eliminated from the gas train.

No iodine was liberated under these conditions. It was found, however, that the addition of thiosulfate to the completely deaerated reaction mixture resulted in a slow disappearance of the typical orange-red color of B_{12a} and the formation of a yellow-brown color similar to that of B_{12r} obtained by hydrogenation. The B_{12a} color was readily restored on the introduction of air, and the formation of free iodine rapidly followed.

3. *The Catalytic Action of B_{12a} on the Oxidation of Iodide by Air. Effect of Varying Conditions.* A solution of potassium iodide, containing also the starch indicator, was placed in a 500 ml. wide-mouth, conical flask equipped with a three-hole rubber stopper through which passed a gas inlet tube, buret tip, and outlet tube. The solution was stirred vigorously magnetically. The gas inlet tube ended in a fritted glass dispersion cylinder. The solution was deaerated for 20 minutes with oxygen-free nitrogen. Oxygen-free sulfuric acid was then pipetted into the flask through the outlet tube. The B_{12a} solution was then added. The flow of nitrogen was stopped, a stream of air was started through the solution, and zero time was taken. The air was delivered under constant pressure, atmospheric pressure plus 5 cm. of mercury, obtained by the usual T-tube pressure regulator. After 5.0 minutes, the air stream was replaced abruptly by a stream of nitrogen. This was continued 5.0 minutes. The free iodine was then titrated with standard thiosulfate, the latter being stored under oxygen-free nitrogen in a Machlett buret and delivered to the reaction vessel without exposure to air. A small stream of nitrogen was continued during the titration.

The total volume in each reaction was 320 ml. In the course of the study, the quantities of B_{12a}, potassium iodide, and sulfuric acid were varied as described below. Ten ml. of a 1 per cent solution of starch was added in each case. The sodium thiosulfate solution (0.0095 N) was standardized by titrating aliquots of a standard solution of potassium

iodate treated with excess potassium iodide and hydrochloric acid; this thiosulfate solution remained constant in concentration for over a month. A 1 per cent solution of starch served as indicator.

The experiments were all run at room temperature, 27° ± 2°. No closer attention was paid to temperature inasmuch as a larger source of error is inherent in the method; see below under results. In several experiments the gases leaving the reaction vessel were bubbled through a solution of potassium iodide; no iodine was collected in this trap in any of the runs.

(a) Variation of B_{12a} Concentration. In each reaction mixture, the solution contained 10.00 ml. of 4.357 N sulfuric acid (final concentration: 0.136 N), 10.00 g. of potassium iodide, and 10.0 ml. of 1 per cent starch. The amount of B_{12a} (aliquots of solutions standardized spectrophotometrically) varied from 11 μg. to 448 μg. The total volume of the solution was 320 ml. The results of this series of experiments are shown in Figure 1.

(b) Variation of Potassium Iodide Concentration. In each reaction mixture was placed 79.0 μg. of B_{12a}, 10.00 ml. of 4.357 N sulfuric acid, and 10.0 ml. of 1 per cent starch. The amount of potassium iodide was varied from 2.0 to 35.0 g. The results are shown in Figure 2.

(c) Variation of Sulfuric Acid Concentration. In each reaction mixture was placed 122 μg. of B_{12a}, 10.00 g. of potassium iodide, and 10.0 ml. of 1 per cent starch. The concentration of sulfuric acid in the final solution was varied from 0.0136 N to 0.272 N. The results are shown in Figure 3.

(d) Effect of Aging the B_{12a} Solution. Crystalline B_{12a} was dissolved in water, and aliquots of the solution were taken at intervals for spectrophotometric measurement and for a determination of its catalytic effect. The spectrophotometric measurements were made on a solution buffered at *p*H 7.4 with 0.2 M phosphate. The conditions for catalysis determinations were held constant: 320 ml. total volume, 10.0 ml. of 4.357 N sulfuric acid, 10.0 g. of potassium iodide, 10 ml. of 1 per cent starch, 5.0 minutes aeration, and 5 minutes sweeping with nitrogen.

4. *B_{12} as Catalyst.* Using the same conditions given above in (a), B_{12} was substituted for B_{12a}. Only very small amounts of iodine were liberated. An appreciable amount of iodine was liberated when a beam of light from a carbon arc was directed into the flask.

5. *The Reduction of B_{12a} by Thiosulfate. Titration of B_{12r}.* An excess of standard soduim thiosulfate was added to an oxygen-free solution of 2.62 mg. of B_{12a} and 10 ml. of 4.0 N sulfuric acid. On standing, the solution changed in color from orange-red to yellow-brown. After 3 hours the solution was titrated potentiometrically with iodine. The iodine required was equal to that required for the titration of the thiosulfate alone. The reduction of B_{12a} by thiosulfate is reversible in agreement with the observation reported in section 2 (last paragraph) above. Thiosulfate is a stronger reducing agent than B_{12r} and both are oxidized by the iodine.

A solution of B_{12r} was prepared by the catalytic hydrogenation of B_{12} using the apparatus of Diehl and Murie (3). An aliquot of this solution containing 4.34 mg. of B_{12}, was transferred to an oxygen-free solution containing 10 ml. of 4.0 N sulfuric acid in 200 ml. of water. The

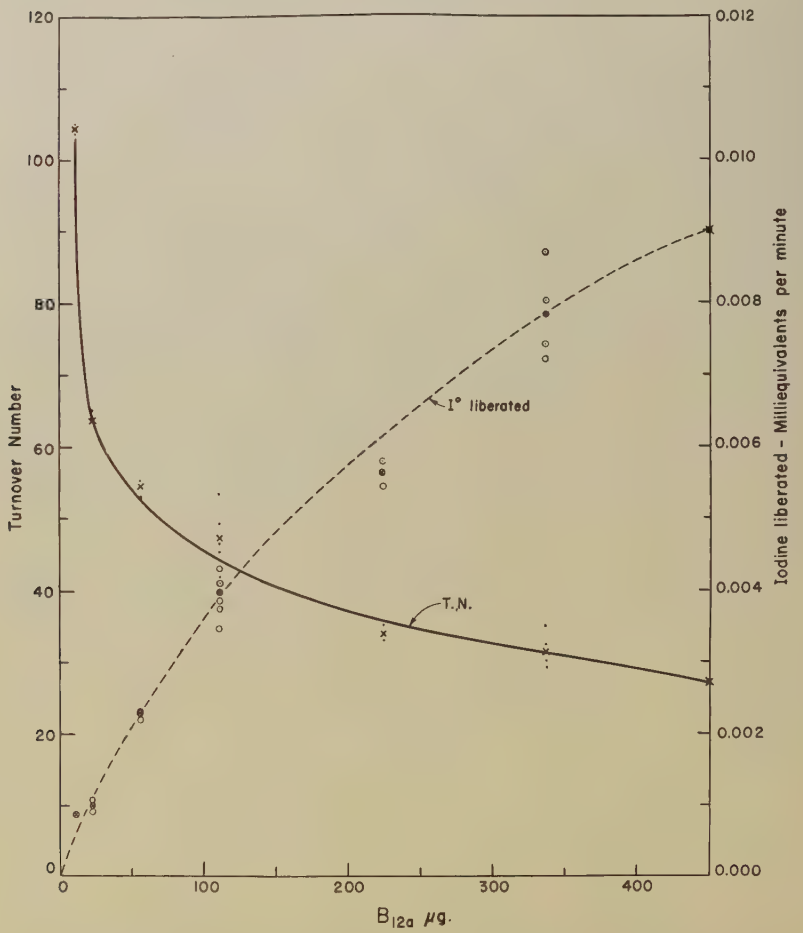

FIG. 1. Catalytic oxidation of iodide by air as a function of the amount of B_{12a} present.

B_{12r} was then titrated potentiometrically with standard iodine. A smooth titration curve was obtained, one equivalent of oxidizing agent per mole of B_{12r} being required. The potential at the mid-point of the titration was +0.25 volts on the hydrogen scale. This is higher than the +0.09 volts found by Diehl and Murie (3) in the titration of B_{12r} with ferricyanide

in neutral solution, the difference undoubtedly resulting from a difference in the acidity of the solution.

RESULTS

From the volume and concentration of thiosulfate used and the

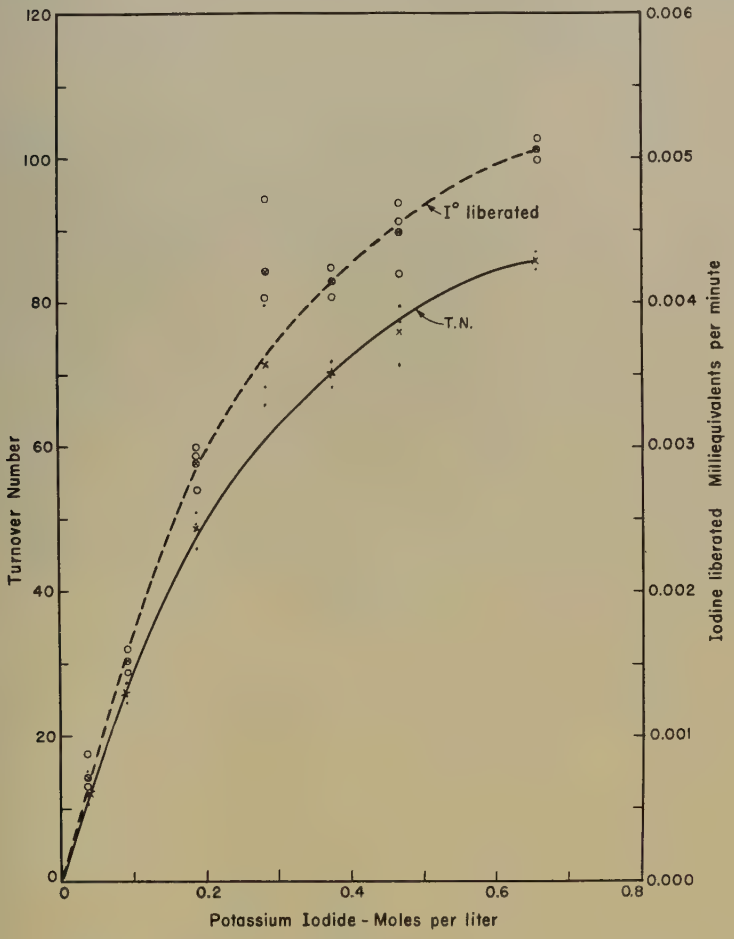

FIG. 2. Catalytic oxidation of iodide by air as a function of potassium iodide concentration.

time of the reaction, the velocity of the reaction was calculated as milliequivalents of iodine liberated per minute. The turnover numbers reported were obtained by dividing the velocity by the number of millimoles of B_{12a} present, the molecular weight of B_{12a} being taken as 1350.

The effect of B_{12a} concentration on the velocity of the reaction and on the turnover number is shown in Figure 1. The velocity increases as the concentration of B_{12a} increases. The turnover number, however, decreases with increasing amounts of B_{12a}. The failure of the velocity to maintain a uniform increase, and the decrease in the turnover number, at higher concentrations of B_{12a} probably results from the lack of sufficient oxygen to maintain maximum activity at higher concentrations of B_{12a}.

The values reported for velocity and turnover number show considerable variation, as much as twenty per cent between the high and low observations of duplicate experiments in one instance. This resulted largely because the method of quenching the reaction was not entirely satisfactory; that is, the displacement of oxygen from the solution by a stream of nitrogen does not stop the reaction sharply. Auxiliary experiments showed that the reaction continued approximately three minutes after the air stream was replaced by nitrogen. The time intervals and rates of gas flow and stirring were held constant in all of the runs however.

As seen from Figures 2 and 3, velocity and turnover number increase with increasing concentration of potassium iodide and with increasing concentration of acid. In a neutral solution, *p*H 7, phosphate buffer, no oxidation of iodide occurred at all.

Data showing the change in the position of the absorption peak and in absorbancy, together with turnover numbers as a function of time, are shown in Table 1. The differences in turnover number are of the order of accuracy of the experimental work and indicate that no appreciable variation in catalytic power of B_{12a} occurs on aging.

DISCUSSION

VITAMIN B_{12a} AS AN OXYGEN CARRIER

Oxygen-carrying cobalt compounds have been described, notably the derivatives of disalicylalethylenediimine (4), of a cobaltous chloride-ammonia-ammonium chloride mixture (5), and the cobaltous derivatives of histidine (6). That B_{12a}, which results from the action of oxygen on the bivalent cobalt compound B_{12r}, might be a reversible, oxygen-carrier is not inconceivable. As such, it should contain molecular oxygen in the form of a peroxo group linking together two cobalt atoms. Such peroxo groups react with iodide to form free iodine. The failure of B_{12a} to liberate iodine, as shown experimentally above, leads us to believe that B_{12a} does not possess a peroxo linkage. Several other observations which bear on this subject are worth recording.

The diamagnetic character of B_{12a} can be interpreted either as trivalent cobalt or as oxygenated bivalent cobalt (7). The passage of oxygen-free nitrogen through a solution of B_{12a} at room temperature does not effect a change in the absorption spectrum of B_{12a}, either in the visible or the ultraviolet, as might be expected if oxygen were being re-

moved and brown B_{12r} re-forming. Moreover, the characteristics of the polarograph wave of B_{12a} are not changed on the addition or removal of oxygen from the solution.

A peroxo group present in B_{12a} would be expected to pass to molecular oxygen on the conversion of B_{12a} to B_{12} by treatment with cyanide.

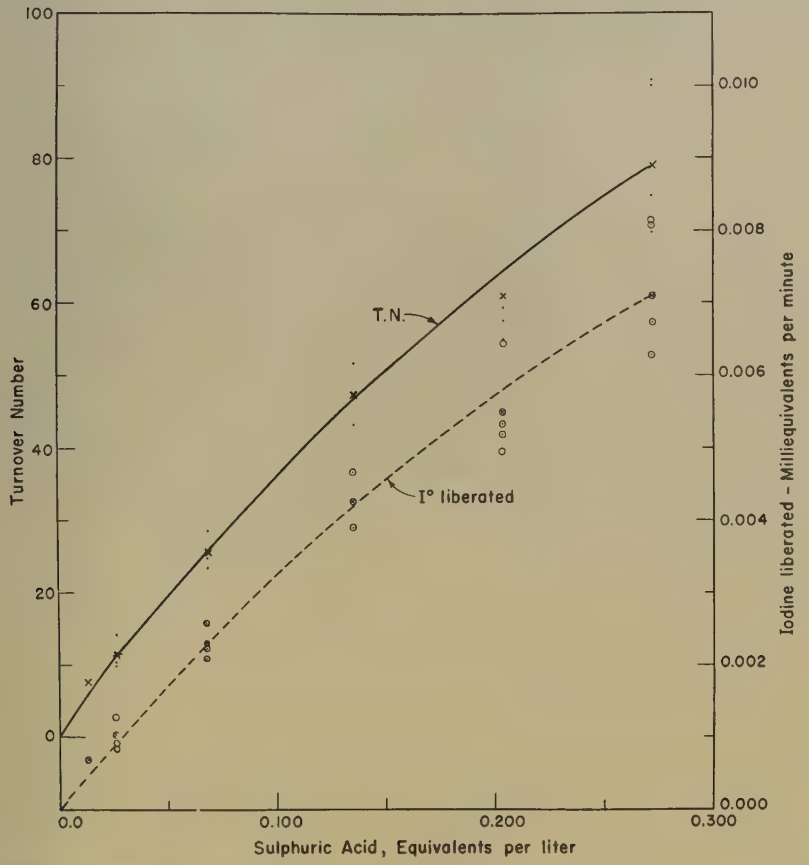

FIG. 3. Catalytic oxidation of iodide by air as a function of sulfuric acid concentration.

No evolution of gas occurs during this conversion, however, providing further support for the absence of a peroxo group.

The molecular weight of vitamin B_{12a} has not been determined. Direct methods for its measurement involving aqueous solutions are unlikely to succeed owing to the changes B_{12a} undergoes when dissolved in aqueous media. Indirect evidence from measurements of the diffusion

constants (8) leads to a value twice as great as for B_{12}, although the value obtained for B_{12} is seriously in error (550 instead of 1350), probably because of departures from the conditions assumed in applying the Stokes-Einstein equation.

Crystalline B_{12a} dissolved in water undergoes a change which is approximately 50 per cent complete at the end of the first day and nearly complete after ten days. The electrical conductance increases, the *p*H rises, and the absorption peak in the neighborhood of 355 mμ is shifted

TABLE 1

VARIATION IN ABSORPTION MAXIMUM, ABSORBANCY AND TURNOVER NUMBER OF B_{12a} SOLUTION ON STANDING*

Time *Hours*	Absorption Maximum mμ	Maximum Absorbancy	Turnover Number Equiv. I° per min. per mole of B_{12a}
0.5	357–58	0.374	73.5
2.5	356–58	.362	64.8
5.2	356–57	.356	64.2
7.2	355–56	.353	65.4
11.2	354–56	.354	48.1
13.5	354–55	.356	62.8
19.3	354–55	.354	67.2
25.6	353–55	.357	60.4
31.0	353–55	.357	70.8
36.0	353–55	.360	63.4
48.0	353–54	.366	64.2
73.0	353	.366	.....

* Phosphate buffers, *p*H 7.4.

toward the lower wave lengths. Inasmuch as only one titratable hydroxyl is present initially and after standing, and because the values for K_b are somewhat different, the change is quite probably the replacement of a hydroxyl group in the coordination sphere about the cobalt atom by a molecule of water. This has already been suggested by the British Drug House group (9). It might well be expected that the catalytic effect on the air oxidation of iodide would depend on the extent of this change after dissolution of the B_{12a} crystals. As indicated above, however, experiments showed no significant variation with time.

THE CATALYTIC BEHAVIOR OF B_{12a}

The variation in the velocity of the oxidation of iodide to free iodine by air with changing iodide concentration, Figure 2, resembles similar

plots for enzymatic reactions. A plot of reciprocal velocity against reciprocal iodide concentration yielded a straight line, Figure 4, in agreement with simple enzyme theory (10). The value obtained for the dissociation constant of the B_{12a}-I^- complex (enzyme-substrate complex), $K_S = 0.396$ M, is large in comparison with K_S values of most enzyme-substrate complexes.

The over-all reaction, of course, involves the hydrogen ion, $O_2 + 4H^+ + 4I^- = 4I^\circ + 2H_2O$, so that it is not surprising to find the reaction dependent on the hydrogen ion concentration. A plot of reciprocal

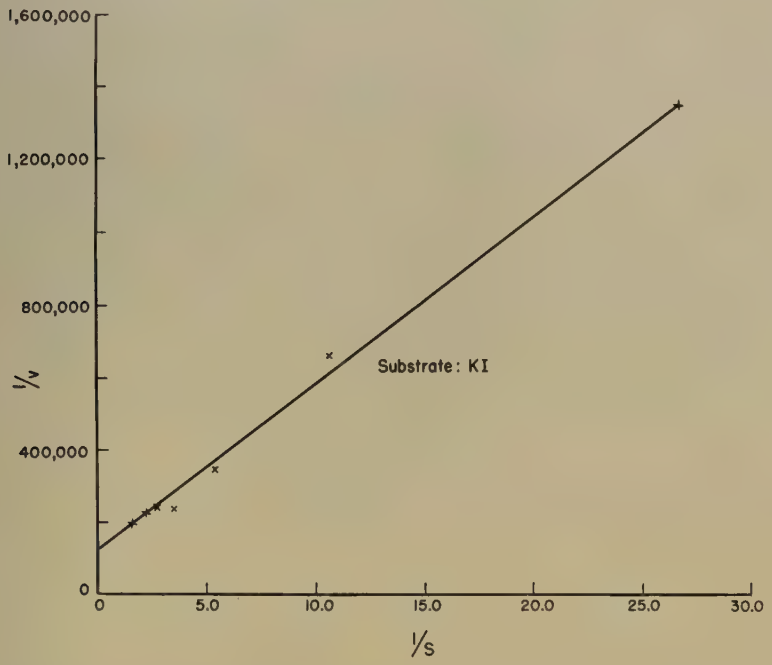

FIG. 4. Catalytic oxidation of iodide by air; plot of reciprocal velocity, v, (moles per liter per minute) against reciprocal iodide concentration, S, (moles per liter). Calculated from data of Figure 2. $K_s = 0.40$ M; $V = 8.3 \times 10^{-6}$ moles I° per minute (maximum velocity).

velocity against reciprocal acid concentration gave a straight line indicating that only one hydrogen ion enters into the rate controlling step, Figure 5. Considering sulfuric acid as the substrate, the dissociation constant has the value, $K_s = 0.430$ M.

Cyanide acts as an inhibitor for this enzymatic system, for B_{12} which is formed by the addition of cyanide to B_{12a} is inactive as a catalyst. It has been demonstrated that cyanide is detached from B_{12} by irradiation with ultraviolet light (11), and this is in accord with the catalysis observed when the iodide-sulfuric acid-air-B_{12} solution was irradiated.

Using the technique employed in this work, it is impossible to study this inhibition quantitatively, for hydrogen cyanide would be rapidly swept away from an acid solution by the air stream passing through the solution.

Preliminary attempts to obtain oxidation under more nearly physiological conditions were performed by including several pure proteins in the reaction mixture with the objective of providing a carrier which might change the conditions of acidity under which the catalysis by

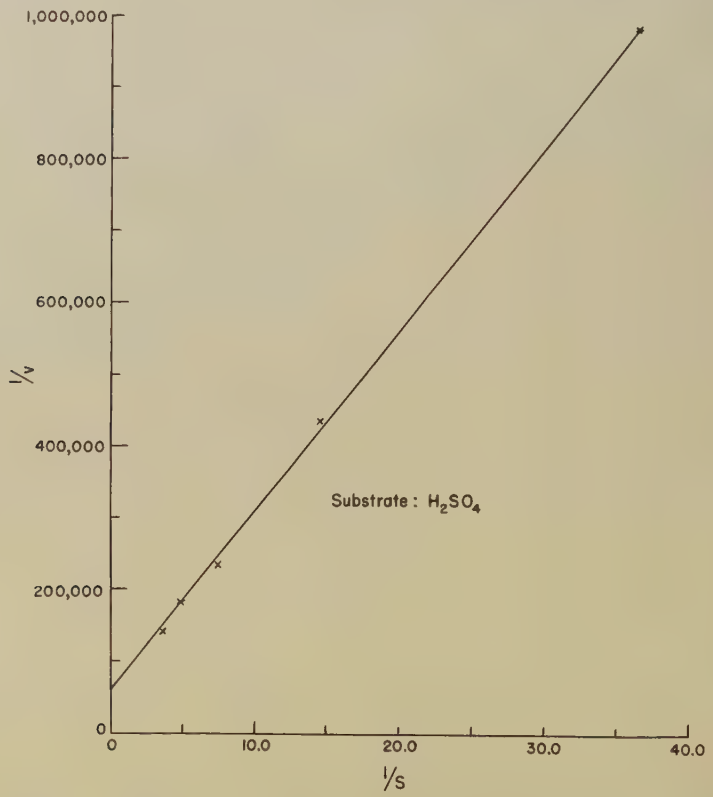

FIG. 5. Catalytic oxidation of iodide by air; plot of reciprocal velocity, v, (moles per liter per minute) against reciprocal sulfuric acid concentration, S, (equivalents per liter). Calculated from data of Figure 3. $K_s = 0.43$ M; $V = 1.7 \times 10^{-5}$ moles of I° per minute (maximum velocity).

B_{12a} occurs. No significant oxidation occurred, even in periods of time up to 25 minutes and *p*H values down to 2.5. Nor did histidine activate the catalyst in neutral solution. It seems quite possible, however, that B_{12a} might have physiological significance in oxidative processes involving iodine.

SUMMARY

Vitamin B_{12a} catalyzes the oxidation of iodide to free iodine by air in acid solution.

The rate at which the air oxidation of iodide occurs was measured at various concentrations of B_{12a}, potassium iodide and sulfuric acid.

The system resembles an enyzmatic system inasmuch as plots of the reciprocal velocity versus reciprocal substrate concentration yield a straight line, considering potassium iodide and sulfuric acid as substrates. Values for the dissociation constant of the enzyme-substrate complex were obtained.

Vitamin B_{12} does not catalyze the air oxidation of iodide and therefore cyanide may be considered as a poison in this system.

It was postulated that the catalytic behavior of vitamin B_{12a} involves the addition of molecular oxygen to the bivalent cobalt atoms of B_{12r}, forming a binuclear compound in which two cobalt atoms are linked through a peroxo group. Vitamin B_{12a}, however, under completely oxygen-free conditions failed to oxidize iodide in acid solution and therefore cannot, itself, contain a peroxo group.

Other information relative to the nature of B_{12a} is presented.

ACKNOWLEDGMENT

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THE NATURE OF THE SPARING PHENOMENON

III. Agglutination of Duck Erythrocytes by Chicken Plasma Potentiated With Alcoholic Extract of Horse Kidney; *in Vivo* Tests[1]

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It has been shown (1) that the hemagglutinin titer in duck erythrocyte-fresh chicken plasma reactions is usually decreased in the presence of duck organ (liver, etc.) extract or duck plasma seromucoid, but in a few instances it is increased. Since the duck organ extract was prepared by Brunius' (2) method for obtaining "source material" of the Forssman hapten from horse kidney, a study of the possible identity of hemagglutinin-inhibitor and Forssman substance was indicated. The work which led to identifying hemagglutinin-inhibiton with the sparing phenomenon (3) suggested the *in vivo* tests carried out on chicks in this study. Jacobs (4) has reported the negative results of attempts to increase resistance of ducklings to *Plasmodium lophurae* by preliminary treatment with materials containing the Forssman hapten.

METHODS

Fresh kidneys obtained from horses sacrificed in the veterinary clinic were brought to the laboratory and, after removal of visible fat, were finely ground. The tissue was subjected to acetone and alcohol extractions as in the case of duck liver (1). The final product in aqueous solution, henceforth called HKE (horse kidney extract), was preserved at —20°C. One cc. of HKE was equivalent to 1 g. of kidney. The procedure employed in the *in vitro* tests for comparing hemagglutinin titers in the presence of physiological salt solution and of HKE was patterned after previous tests (1, 5). The experiments were carried out at ordinary room temperatures. The donors of the chicken plasma varied from four to nine months of age. Chicken plasma and HKE dilutions were thoroughly mixed and allowed to react for about two hours before the 1:50 duck erythrocyte suspension ("duck RBC") was added. Degrees of agglutination recorded in tables were read at the time of greatest differences observed.

[1] This investigation was supported (in part) by grants from the Industrial Science Research Institute, Iowa State College, and the Division of Research Grants and Fellowships of the National Institute of Health, United States Public Health Service.

The cooperation of Drs. G. R. Fowler, M. A. Emmerson, E. A. Benbrook, W. H. Chivers, and F. K. Ramsey of the Veterinary Division in obtaining the fresh horse kidneys is gratefully acknowledged.

The procedure for studying the effect of HKE on duck cells parasitized with *P. lophurae* in the blood stream of chicks 8–11 days of age was also patterned after that employed in previous tests (3, 6, 7). HKE was injected into a wing vein of the chick about an hour before the parasitized duck erythrocytes.

RESULTS

Numerous tests were made with HKE prepared from kidneys of five horses. Positive potentiating effects of HKE on agglutination of duck erythrocytes by fresh chicken plasmas were observed in the case of 29 of 40 plasmas tested. Five of the plasmas did not agglutinate duck erythrocytes at concentrations up to 30 per cent, and six of them that did agglutinate did not exhibit the potentiating effect when supplemented with HKE. None of the chick plasmas employed had been frozen, because freezing usually increases the titer (1), and the titers of such enhanced plasmas, as was observed repeatedly, cannot be further potentiated with HKE.

The pertinent data for an experiment in which the combinations of chicken plasma and HKE were strikingly reactive appear in Table 1. In this instance the potentiating effect was observable when HKE constituted but one part in 2560. Titers of 2560 were unusual; most of them were between 16 and 256. The optimum amount of chicken plasma to be used was found to be 0.2 cc., for this amount definitely agglutinated duck erythrocytes (tubes 5, 12, 21), while half as much (tube 22) barely agglutinated them. In general, chicken plasmas which did not agglutinate at least feebly in amounts of 0.5, 0.1, 0.2 or 0.3 cc. would not agglutinate when supplemented with HKE, nor would dilutions of agglutinating plasmas which were too weak to agglutinate on standing for several hours, or which agglutinated feebly. The various dilutions of HKE (tubes 2, 4, 7, 9, 11, 14, 16, 20) did not of themselves possess the power of hemagglutination.

In Table 2 appear the pertinent data for an experiment testing the effect of duration of interaction between a plasma known to possess hemagglutinating properties and various concentrations of a 1:4 dilution of HKE. The readings for each strength of plasma were all made after the same time interval, about eight minutes after the erythrocyte suspension was added, beginning with the four-minute series. This chicken plasma did not agglutinate well in 0.1-cc. amounts either by itself or supplemented with HKE. In 0.2-amounts, however, it definitely agglutinated at 4 minutes, and the reaction progressed over a period of 15 hours.

There were two chickens (A and B) of the 40 whose plasma consistently showed "prezoning" with the duck cells available; i.e., the stronger concentrations showed weaker agglutinations than the weaker concentrations, up to a certain point. Comparisons of the degree of agglutination in tube 18 of Table 3, Part 1, with that in tube 12, and that in tube 12 with that in tube 6 are good illustrations of the phenomenon.

Part 1 and Part 2 of Table 3 record the results of testing the effect of four dilutions of HKE on hemagglutination in the presence of several concentrations of plasmas that exhibited prezoning. The table shows the following points clearly: (1) The degree of agglutination by the two untreated chicken plasmas decreased as their concentration increased;

TABLE 1

DEGREE OF AGGLUTINATION OF DUCK ERYTHROCYTES BY CHICKEN PLASMA IN PRESENCE OF HORSE KIDNEY EXTRACT (HKE)

Tube No.	Plasma Ch. C ♂	0.85 Per Cent NaCl	HKE		Duck RBC	Degree of Agglutination
			Conc.	Amt.		
1	0.2	0.4	1/1	0.2	0.2	XX(−)
2	.....	0.6	1/1	0.2	0.2	0
3	0.2	0.5	1/1	0.1	0.2	XXX(+)
4	.....	0.7	1/1	0.1	0.2	0
5	0.2	0.6	.....	.....	0.2	X
6	0.2	0.5	1/2	0.1	0.2	*
7	.....	0.7	1/2	0.1	0.2	0
8	0.2	0.5	1/4	0.1	0.2	*
9	.....	0.7	1/4	0.1	0.2	0
10	0.2	0.5	1/8	0.1	0.2	*
11	.....	0.7	1/8	0.1	0.2	0
12	0.2	0.6	.....	.....	0.2	X
13	0.2	0.5	1/16	0.1	0.2	*
14	.....	0.7	1/16	0.1	0.2	0
15	0.2	0.5	1/32	0.1	0.2	*
16	.....	0.7	1/32	0.1	0.2	0
17	0.2	0.5	1/64	0.1	0.2	*
18	0.2	0.5	1/128	0.1	0.2	*
19	0.2	0.5	1/256	0.1	0.2	X(+)
20	.....	0.7	1/256	0.1	0.2	0
21	0.2	0.6	.....	.....	0.2	X
22	0.1	0.7	.....	.....	0.2	±
23	0.3	0.5	.....	.....	0.2	X(+)

* Tubes 6, 8, 10, 13, 15, 17, and 18 showed an almost perfectly graded series of decreasing degrees of agglutination between XXX(+) of tube 3 and X(+) of tube 19.

(2) in the case of each concentration of plasma, except 0.1 cc. of chick A, treatment with HKE increased the degree of agglutination very strikingly; (3) the degree of agglutination by the treated plasmas increased with the concentration of the plasmas. The evidence for the latter is the comparison of the agglutinations in tubes 2–5, tubes 8–10, and tubes 14–16 of Part 1 and in tubes 1, 2, 4, 5, tubes 6, 7, 9, 10, and tubes 11, 12 14, 15 of Part 2.

In tubes 2–5 of Part 1 the potency of the chicken plasma was slightly reduced by HKE, and in tubes 1, 2, 4, 5 of Part 2 it was not affected. Similar inhibitions have been observed in other instances with either

TABLE 2

Degree of Agglutination of Duck Erythrocytes by Chicken Plasma in Presence of Horse Kidney Extract (HKE) for 15-hr., 2-hr., 1-hr., 15-min., and 4-min. Intervals

Tube No.	Plasma Ch. D ♀	0.85 Per Cent NaCl	HKE (1/4)	Duck RBC	Degree of Agglutination				
					15 hr.	2 hr.	1 hr.	15 min.	4 min.
1	0.1	0.60	0.10	0.2	±	±	±	±	0
2	0.1	0.50	0.20	0.2	+	±	±	±	0
3	0.1	0.70	.....	0.2	±	±	±	±	0
4	0.1	0.65	0.05	0.2	+	+	+	±	0
5	0.2	0.50	0.10	0.2	XXXX	XXX	XX	X	X(−)
6	0.2	0.40	0.20	0.2	XXXX	XXX	XX	X	X(−)
7	0.2	0.60	.....	0.2	X	X	X(−)	X	X(−)
8	0.2	0.55	0.05	0.2	XXXX	XXX	XXX	X	X(−)

low concentrations of plasma or strong concentrations of HKE. Too strong a concentration of HKE often gels the chicken plasma.

The retarding influence of duck plasma on the agglutination of duck

TABLE 3

DEGREE OF AGGLUTINATION OF DUCK ERYTHROCYTES BY CHICKEN PLASMA IN PRESENCE OF HORSE KIDNEY EXTRACT (HKE), WHEN PREZONING OCCURS

PART 1

Tube No.	Plasma Ch. A ♂	0.85 Per Cent NaCl	HKE (1/1)	Duck RBC	Degree of Agglutination
1	0.1	0.70	.....	0.2	X(+)
2	0.1	0.65	0.05	0.2	X
3	0.1	0.60	0.10	0.2	X
4	0.1	0.50	0.20	0.2	X
5	0.1	0.20	0.50	0.2	X
6	0.1	0.70	.....	0.2	X(+)
7	0.2	0.60	.....	0.2	X
8	0.2	0.55	0.05	0.2	XX
9	0.2	0.50	0.10	0.2	XX
10	0.2	0.40	0.20	0.2	XX
11	0.2	0.10	0.50	0.2	gelled
12	0.2	0.60	.....	0.2	X
13	0.3	0.50	.....	0.2	±
14	0.3	0.45	0.05	0.2	XXX
15	0.3	0.40	0.10	0.2	XXX
16	0.3	0.30	0.20	0.2	XXX
17	0.3	.....	0.50	0.2	gelled
18	0.3	0.50	.....	0.2	±

PART 2

Tube No.	Plasma Ch. B ♀	0.85 Per Cent NaCl	HKE (1/1)	Duck RBC	Degree of Agglutination
1	0.1	0.6	0.1	0.2	XXX
2	0.1	0.5	0.2	0.2	XXX
3	0.1	0.7	.....	0.2	XXX
4	0.1	0.4	0.3	0.2	XXX
5	0.1	0.2	0.5	0.2	XXX
6	0.2	0.5	0.1	0.2	XXXX (−)
7	0.2	0.4	0.2	0.2	XXXX (−)
8	0.2	0.6	.....	0.2	XX
9	0.2	0.3	0.3	0.2	XXXX (−)
10	0.2	0.1	0.5	0.2	XXXX (−)
11	0.3	0.4	0.1	0.2	XXXX
12	0.3	0.3	0.2	0.2	XXXX
13	0.3	0.5	.....	0.2	±
14	0.3	0.2	0.3	0.2	XXXX
15	0.3	.....	0.5	0.2	XXXX

erythrocytes by certain (fresh and unfrozen) chicken plasmas has been reported (3, 6, 7). A comparison (Table 4) of the readings for six chicken plasmas in line A with those in line C illustrates the hemagglutinin-inhibition phenomenon. A comparison of readings for the same six plasmas in line A with those in line B illustrates again the potentiating effect of HKE which has been discussed above. The problem of which reagent, duck-plasma inhibitor or horse-kidney potentiator, would dominate naturally presented itself. One cc. of HKE followed by one cc. of duck plasma was added to each tube in the test with the six plasmas, and the mixtures shaken immediately in the mechanical shaker. A comparison of the readings in line D with those in lines A, B, and C shows that in the presence of duck plasma, HKE was not only unable to assert its intrinsic potentiating properties (line B), but it was also unable to deter duck plasma from exerting all or a large proportion of its inhibiting influence (lines C and D). In the case of the plasma of chick H only, did duck plasma in the presence of HKE fail to hold the intensity of the agglutination down to that produced by the unsupplemented plasma, or below, and here the difference was very slight.

Since the *in vitro* potentiating effect of HKE had been observed in the case of almost three-fourths of the chicken plasmas tested, it was hoped that it would be possible to demonstrate some degree of inhibition of the development of *P. lophurae* by injecting the chick hosts intravenously with HKE. The possibility of obtaining such an *in vivo* effect was considered good in view of the fact that the sparing phenomenon had been produced in chicks by injecting them with duck plasma or duck seromucoid that had exhibited hemagglutinin-inhibition *in vitro* (3). Series 1 and 2 test birds received HKE at the rate of 1.0 cc./100 g. body weight daily beginning an hour before injection; those of series 3 and 4, 2.0 cc./100 g. body weight.

The results of the four series of tests involving 52 test birds and 53 controls are recorded in Table 5. A careful inspection of the data for test and control groups which takes standard deviations into consideration leads only to the conclusion that the injected HKE did not influence significantly the course of *P. lophurae* infection in the chicks.

DISCUSSION

The horse kidney extract (HKE) which exhibited the potentiating effect in mixtures of duck erythrocytes and dilutions of chicken plasma was, as previously stated, prepared by a method which has been used for obtaining the so-called Forssman hapten. The latter substance prepared from guinea pig or horse kidney is known to inhibit hemolysis of sheep cells by the serum of rabbits immunized to sheep cells and to fix complement in complement fixation tests utilizing sheep cells and anti-rabbit serum (Brunius, 2). Hence its role in potentiating hemagglutinin was unexpected. As shown in Table 1, HKE by itself did not agglutinate chicken red cells. The reaction between HKE and agglutinating plasma is protracted, and requires between 2 and 15 hours for its completion.

TABLE 4

DEGREE OF AGGLUTINATION OF DUCK ERYTHROCYTES BY SIX FRESH AND UNFROZEN CHICKEN PLASMAS IN PRESENCE OF SUPPLEMENTS OF (A) SALINE, (B) HKE, (C) DUCK PLASMA, AND (D) DUCK PLASMA AND HKE

Components Reacting With Duck RBC	Source of Chicken Plasma					
	Ch. E ♀	Ch. F ♂	Ch. G ♂	Ch. H ♂	Ch. I ♀	Ch. J ♂
A. Chicken plasma (0.2cc)	XX	XX	±	X	XXX	XX
B. Chicken plasma (0.2cc) + HKE (0.1cc)	XXX	XXX	+	XXX	XXXX	XXXX
C. Chicken plasma (0.2cc) + duck plasma (0.1cc)	±	±	0	±	XX	±
D. Chicken plasma (0.2cc) + duck plasma (0.1cc) + HKE (0.1cc)	±	±	±	X(+)	XX	+

Certain fresh chicken plasmas exhibit the prezoning effect; e.g., 0.3 cc. of such a plasma may show less potency in agglutinating duck red cells than 0.2 cc., and 0.2 cc. in turn less than 0.1 cc., etc. Additions of appropriate amounts of HKE to such dilutions of chicken plasma potentiates hemagglutination so that a higher concentration shows a higher degree of agglutination than a lower concentration. This unmasking of the true hemagglutinin potency of the chicken plasma by HKE is the true explanation of the potentiating effect. As is shown in Table 3,

TABLE 5

MEAN PERCENTAGE OF INFECTED CELLS WITH STANDARD DEVIATIONS DURING INFECTION WITH *Plasmodium lophurae* IN TEST CHICKS RECEIVING HKE AND CONTROLS RECEIVING EQUIVALENT AMOUNTS OF SALINE SOLUTION

(Number of chicks in each group appears at bottom of column.)

Time After Inoculation	Series 1		Series 2		Series 3		Series 4	
	Test	Control	Test	Control	Test	Control	Test	Control
3 min.	0.23 ±0.15	0.32 ±0.27	0.31 ±0.06	0.35 ±0.15	.....	.....	.....	.....
1 hour	.....	.....	.....	.....	0.2 ±0.21	0.04 ±0.05	0.84 ±0.32	0.77 ±0.32
1 day	0.05 ±0.08	0.11 ±0.21	0.04 ±0.08	0.07 ±0.09	0.14 ±0.14	0.07 ±0.06	0.65 ±0.71	0.86 ±0.72
2 days	.....	.....	.....	.....	0.5 ±0.36	0.22 ±0.17	0.84 ±0.99	1.04 ±0.84
3 days	0.3 ±0.59	0.4 ±0.81	0.13 ±0.18	0.36 ±0.37	.....	.....	4.9 ±5.2	6.1 ±5.1
4 days	0.64 ±0.96	1.1 ±2.1	0.2 ±0.32	0.68 ±0.84	6.7 ±5.5	2.9 ±1.6	20.5 ±20.0	20.5 ±17.0
5 days	0.74 ±1.31	0.83 ±1.19	.....	.....	.....	.....	27.5 ±23.7	27.8 ±20.7
6 days	1.2 ±1.7	1.13 ±1.6	3.4 ±5.7	7.0 ±8.7	29.0 ±6.6	24.5 ±8.9	49.1 ±21.7	43.3 ±22.1
7 days	0.64 ±1.25	1.06 ±1.48	5.9 ±7.7	11.1 11.3	25.4 ±13.7	29.4 ±13.9	29.1 ±17.4	19.3 ±14.9
8 days	1.8 ±4.8	3.6 ±6.4	18.1 ±20.8	15.9 ±14.7	38.7 ±25.3	47.9 ±7.5	39.6 ±21.9	19.3 ±26.0
10 days	4.6 ±12.9	8.3 ±16.0	19.0 ±17.7	14.0 ±16.6	22.3 ±20.0	36.6 ±15.3	35.6 ±18.9	8.8 ±12.2
12 days	2.4 ±7.8	3.3 ±6.9	10.9 ±18.6	3.2 ±6.0	11.2 ±18.8	9.5 ±14.1	32.9 ±18.5	7.5 ±13.2
15 days	.....	.....	10.0 ±18.3	2.6 ±6.4	10.8 ±21.2	9.5 ±19.1	11.1 ±11.0	2.1 ±4.7
	(13)	(13)	(12)	(13)	(20)	(20)	(7)	(7)

when enough HKE has been added to produce maximum hemagglutination, additional amounts do not change the picture; but graded lesser amounts, as shown in Table 1, produce corresponding lesser degrees of hemagglutination.

What is the explanation of the unmasking of the full potency of chicken plasma by HKE? It has previously been stated that either sufficient freezing and thawing or heating at 56°C. for 30 min. (1) usually increased the hemagglutinating potency of chicken plasma, providing the plasma already is capable of agglutinating duck cells to a certain degree. It was also noted (1) that, while duck plasma tended to inhibit hemagglutination in the case of most chicken plasmas, a certain few of the latter were actually potentiated by duck plasma. The explanation offered for the observed effects was as follows: (1) Certain chicken plasmas naturally contain a hemagglutinin-inhibitor, possibly in loose union with the hemagglutinin; (2) this inhibitor is destroyed by freezing or by heating at 56°C. for 30 min.; (3) under certain conditions the inhibitor unites with a component of duck plasma, releasing the antibody for agglutinating the duck red cells. A similar explanation would hold also for the potentiating effect of HKE, when substituted for the duck plasma.

That the active components in HKE and duck plasma are not identical is evident from the data in Table 4, where it is shown that, in the case of every one of six chicken plasmas, HKE potentiated hemagglutination while duck plasma inhibited it. It had been learned previously that only occasionally in chicken plasma does duck plasma play a role like that of HKE (1). Table 4 also shows that when agglutinating chicken plasmas are treated simultaneously with both HKE and duck plasma, the latter gives the illusion of playing the dominant role. The obvious explanation is that HKE inhibits the native labile hemagglutinin-inhibitor in chicken plasma, but the duck plasma at the same time inhibits the released innate hemagglutinin, usually more efficiently than the labile hemagglutinin-inhibitor itself. It has been shown that the active component of the duck plasma is the seromucoid (1). There appears (Table 4) to have been little or no reaction between HKE and the active component of the duck plasma.

It was anticipated that when HKE was injected into chicks its potentiating effect would be reflected in increased resistance to introduced parasitized duck cells. It is evident after a study of Table 5 that no consistent results of that sort were obtained. The reason for the negative outcome is not clear, but one possibility is that the organ tissues had a stronger affinity for HKE than plasma, so that it was removed from the blood before its effects could be noted. Series 1 and series 2, admittedly, did show a trend in the direction of the anticipated results, but in neither series were the differences between tests and controls statistically significant. Series 3 and series 4 did not indicate a positive trend.

CONCLUSIONS

1. An extract of horse kidney (HKE) prepared by the method for obtaining the source material of the Forssman hapten exhibited a po-

tentiating (conglutinin) effect on agglutination of duck erythrocytes by chicken plasmas.

2. The effect can be explained by the combination of horse kidney extract with the native labile component of chicken plasma, destructible with heating or freezing, that inhibits its own innate hemagglutinin.

3. Decreasing degree of hemagglutination following increasing concentrations of certain chicken plasmas was occasionally observed.

4. In such plasmas the antihemagglutinating effects increase more rapidly than the concentration of the inhibiting component, because after neutralization of the latter with HKE the hemagglutinin titers of the chicken plasma rise progressively with increasing concentration of the plasma.

5. Since HKE and duck plasma usually play opposite roles in influencing hemagglutination, the active component in duck plasma cannot be the Forssman hapten.

6. The potentiating effect of HKE *in vitro* cannot be translated into increased resistance to duck erythrocytes parasitized with *Plasmodium lophurae* when both HKE and parasitized cells are introduced into the blood stream of the living chick.

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